Investigations éco-épidémiologiques et génétiques des Lyssavirus et des Paramyxovirus chez les micromammifères du Sud-Ouest de l'Océan Indien

THÈSE

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ADN : Acide désoxyribonucléique

AIC : Akaike Information Criterion

AICc: Corrected Akaike Information Criterion

ARN : Acide ribonucléique

BEAST : Bayesian Evolutionary Analysis Sampling Trees

cDNA : Complementary DNA

EID : Emerging Infectious Disease

ESS : Effective sample size values

FAO : Food and Agriculture Organization

GLM: Generalized Linear Model

GLMM: Generalized Linear Mixed Model

GPS: Global positioning system

Kb : Kilobase

MCMC: Markov Chain Monte Carlo

MAT : Mean average temperature

MAR: Mean average rainfall

Mya : Million years ago

OIE : Office international des épizooties, devenu maintenant Organisation mondiale de la santé animale

OTU : Operational taxonomic units

PAR : *Pneumovirus Avulavirus Rubulavirus*

PCR : Polymerase chain reaction

PPR : *Peste des petits ruminants*

PVs: Paramyxoviruses

qRT-PCR : Real time reverse transcriptase polymerase chain reaction

RFFIT : Rapid fluorescent focus inhibition test

RMH: *Respirovirus Morbillivirus Hénipavirus*

RT-PCR : Reverse transcriptase polymerase chain reaction

SOOI: Sud-ouest Océan Indien

SWIO: South Western Indian Ocean islands

UMRVs: *Unclassified Morbilli-Related viruses*

UHRV: Unclassified Henipa-Related viruses

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Résumé

La faune sauvage a été depuis longtemps incriminée dans la survenue de zoonoses et joue le rôle de réservoir d'agents pathogènes (virus *Nipah*, *Hendra*, *Ebola*, *Hantaan* etc.) pour l'homme. Les îles tropicales et subtropicales du Sud-Ouest de l'Océan Indien (SOOI) constituent l'une des 34 régions reconnues comme « *hotspot* » de biodiversité au niveau mondial. Elles sont caractérisées par un très fort endémisme de la faune sauvage surtout sur l'Ile de Madagascar. Le caractère multi-insulaire de la région du SOOI, la diversité de ses biotopes et ses disparités biogéographiques et humaines offrent un champ d'investigation unique pour explorer « *in natura* » la dynamique évolutive des agents infectieux et les relations hôtes-virus.

Nos travaux de recherche ont porté sur deux modèles de virus à ARN de polarité négative, les paramyxovirus et les lyssavirus. Le premier modèle viral nous a permis d'aborder les questions relatives à la dynamique de transmission virale au sein de communauté d'hôtes, plus particulièrement, les chauves-souris et les petits mammifères terrestres de Madagascar et d'identifier les facteurs agissant sur cette dynamique de transmission et de diversification virale, en particulier les facteurs bio-écologiques associés à leurs hôtes. Le second modèle viral, les lyssavirus, nous a permis de décrire sur l'ensemble des îles du SOOI échantillonés, la circulation virale dans ce système multi-insulaire diversifié, au sein des chauves-souris dont la plupart des espèces sont endémiques à cette région.

Dans l'ensemble, nos investigations ont permis de mettre en évidence des échanges viraux (« *host-switch* ») importants entre chauves-souris, petits mammifères terrestres endémiques de Madagascar et les rongeurs introduits, le rôle de ces mammifères en tant que réservoir viral majeur et souligner le rôle disséminateur de *Rattus rattus*. Par ailleurs, nous avons pu identifier ce phénomène de « *host-switch* » comme étant le mécanisme macro-évolutif prépondérant et l'importance des facteurs biotiques et abiotiques à l'origine de la dynamique de transmission et de la diversification virale observée chez les paramyxovirus de chauves-souris de Madagascar.

Mots-clés : faune sauvage, zoonoses, SOOI, biodiversité, lyssavirus, paramyxovirus, diversité virale, réservoir viral, disséminateur viral, « *host-switch* » facteurs écologique

Abstract

For several decades, the animal have been incriminated as an important reservoir of many zoonotic pathogens (*Nipah*, *Hendra*, *Ebola*, *Hantaan* viruses etc.) at risk for humans. Tropical and subtropical islands of the South Western Indian Ocean (SWIO) are comprise on of 34 areas of the world recognized as biodiversity "hotspot". They are characterized by high levels of animal endemism, particularly on Madagascar. The multi-island structure of the SWIO region, the diversity of its biotopes, and differences in aspects related to human culture, offer a unique opportunity to investigate "*in natura*" the evolutionary dynamics of infectious agents and associated host-virus relationships.

Our research has focused on two models of negative RNA viruses, paramyxoviruses, and lyssaviruses. The first virus model allowed us to address issues related to the dynamics of viral transmission within a host community, in particular bats and Malagasy small terrestrial mammals, and to identify the driving factors, especially bio-ecological aspects associated with their hosts, affecting the dynamic of transmission and viral diversification. The second model allowed us to describe on the islands, the intense circulation of bat lyssaviruses in this system, including bats endemic to this region.

Overall, our investigations highlighted: (i) intense viral exchanges ("*host-switch*") between bats, endemic terrestrial small mammals, and introduced rodents on Madagascar, (ii) the role of these mammals as major viral reservoir, and (iii) the key role played by *Rattus rattus* as viral spreader. Furthermore, we identified both the phenomenon of "*host-switch*" as the major macro-evolutionary mechanism among bat paramyxoviruses from Madagascar and the importance of biotic and abiotic factors in shaping the transmission dynamics and viral diversification.

Keywords: wild fauna, zoonoses, SWIO, biodiversity, lyssavirus, paramyxoviruses, viral diversity, reservoir, viral spreader, "*host-switch*" ecological factors

PARTIE I. ETAT DE L'ART

L'état de l'art sera présenté en 3 chapitres:

Dans le chapitre I nous aborderons les notions générales sur les maladies infectieuses émergentes, les principales étapes d'émergences et les facteurs d'émergences dans le but de mieux comprendre et appréhender leur survenue.

Dans le chapitre II, nous nous focaliserons sur les agents zoonotiques retrouvés chez les chauves-souris et les petits mammifères ainsi que l'importance de leurs caractéristiques biologiques dans le maintien et la transmission d'agents zoonotiques.

Dans le chapitre III, nous détaillerons les caractéristiques bio-écologiques de la zone Sud-ouest de l'Océan, notamment la biodiversité résidente en micromammifères, en mettant un accent sur la faune sauvage de Madagascar et particulièrement sur les chauves-souris et les petits mammifères endémiques qui y vivent.

Chapitre 1. Les maladies infectieuses émergentes

1.1. Généralités

L'Organisation Mondiale de la Santé (OMS) définit les maladies infectieuses émergentes comme des maladies survenant pour la première fois dans une population ou qui ont déjà sévi mais dont la prévalence augmente à nouveau à un niveau élevé (ré-émergentes) présentant une grave menace pour les populations exposées. Par le passé, les plus dangereuses d'entre elles ont provoqué chez l'homme des pandémies de choléra, de variole, de rougeole ou encore de grippe causant la mort de millions de personnes dans le monde. Les études récentes ont mis en évidence une augmentation significative du nombre d'émergences infectieuses depuis les années 1940, et des auteurs ont recensé en 2008 plus de 300 maladies infectieuses (Jones et al., 2008). Les maladies qui sont naturellement transmises de l'animal à l'homme et vice versa, appelées zoonoses, constituent plus de la moitié des maladies infectieuses émergentes (Jones et al., 2008). Plus de 75% d'entre elles ont pour origine la faune sauvage (Taylor et al., 2001). Par ailleurs, on sait que plus de 25% de ces maladies seraient dues à des pathogènes viraux (Taylor et al., 2001 ; Jones et al., 2008). A ce jour, il est estimé que sur les 57 millions de morts par an dans le monde, environ 14,9 millions seraient dus aux maladies infectieuses, soit plus de 25% (Muray & Lopez 1996 ; Fauci et al., 2001) (**Figure 1**) avec un impact plus important dans les pays en développement (Guerrant & Blackwood, 1999). On estime à plus de 1400 le nombre d'agents infectieux pathogènes pour l'homme (Taylor et al., 2001). En 2001, des auteurs ont rapporté qu'en moyenne, trois à quatre nouveaux virus pathogènes étaient détectés en population humaine chaque année, et la plupart de ces nouveaux agents provenaient des espèces animales (Woolhouse & Gaunt, 2007).

1.1.1. Exemple d'agent émergent : le virus EBOLA

Le virus *Ebola* de la famille des *Filoviridae*, a récemment ré-émergé entre 2013 et 2015 en Afrique de l'Ouest là où le virus est endémique, provoquant une fièvre hémorragique grave avec une issue fatale fréquente chez les humains, (plus de 11000 décès sur 23000 cas [WHO, 2015]) et plusieurs espèces de primates non humains dont les grands singes.

Les chauves-souris frugivores étaient considérées comme le réservoir naturel du virus Ebola en Afrique (Leroy et al., 2005). Cependant, au cours de l'épidémie survenue en 2015, les chauves-souris insectivores ont été fortement incriminées et leur contact avec l'homme serait à l'origine de la ré-émergence récente du virus (Marí Saéz et al., 2014).

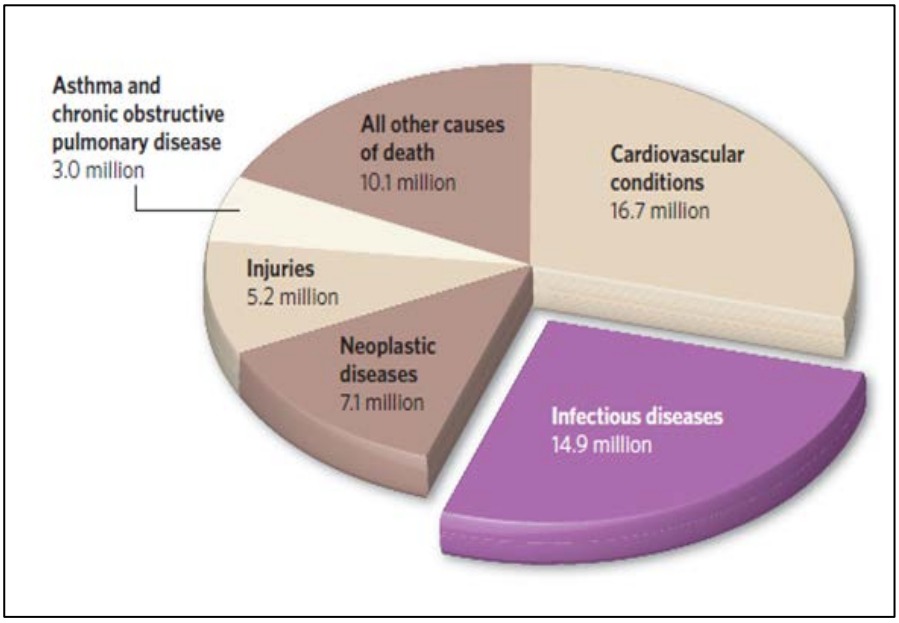

Figure 1. Place des maladies infectieuses parmi les causes de mortalité/morbidité dans le monde (Morens et al., 2004).

1.1.2. Exemple d'agent émergent : le SARS-Coronavirus

Un second exemple saisissant d'émergence concerne le Syndrome Respiratoire Aigu Sévère (SRAS) provoqué par un virus de la famille des *Coronaviridae* qui a émergé dans les populations humaines en Chine en 2003 faisant plus de 8000 cas et plus de 700 décès (WHO, 2014). Des analyses sur des échantillons d'animaux sauvages vendus comme viande de brousse sur des marchés de la province de Guangdong (Chine) ont détecté la présence de coronavirus du SRAS chez des civettes (Guan et al., 2003) et des chauves-souris insectivores (Lau et al., 2005). Un scénario d'émergence a été proposé incriminant un contact indirect entre l'homme et la faune sauvage par l'intermédiaire d'animaux domestiqués, les civettes.

1.1.3. Réservoirs et sauts d'espèces

De nombreux autres virus illustrés sur la **Figure 2** sont des exemples bien documentés de zoonoses qui ont eu aussi, ou menacent d'avoir, un impact sur la santé humaine (Morens et al., 2004).

Le point commun à toutes ces émergences virales reste le franchissement de la barrière d'espèce des virus de la faune sauvage ou domestique, considérés comme des réservoirs, vers la population humaine. Nous utilisons le terme réservoir selon la définition de Drexler et al. comme un taxon animal naturellement infecté de façon durable, au niveau populationnel et au-delà des limites géographiques, par une diversité d'agents infectieux, et pouvant ou non exprimer la maladie (Drexler et al., 2012). L'émergence d'un pathogène dans une espèce nouvelle (et pas uniquement dans la population humaine) va impliquer un saut de celui-ci depuis son hôte originel « réservoir » dans lequel il est déjà établi, vers un hôte différent. Ce phénomène est communément appelé « franchissement ou saut de la barrière d'espèces » (« *host-switch* ») et les mécanismes qui en rendent compte ne sont pas encore très bien compris (Morens et al., 2004 ; Longdon et al., 2011).

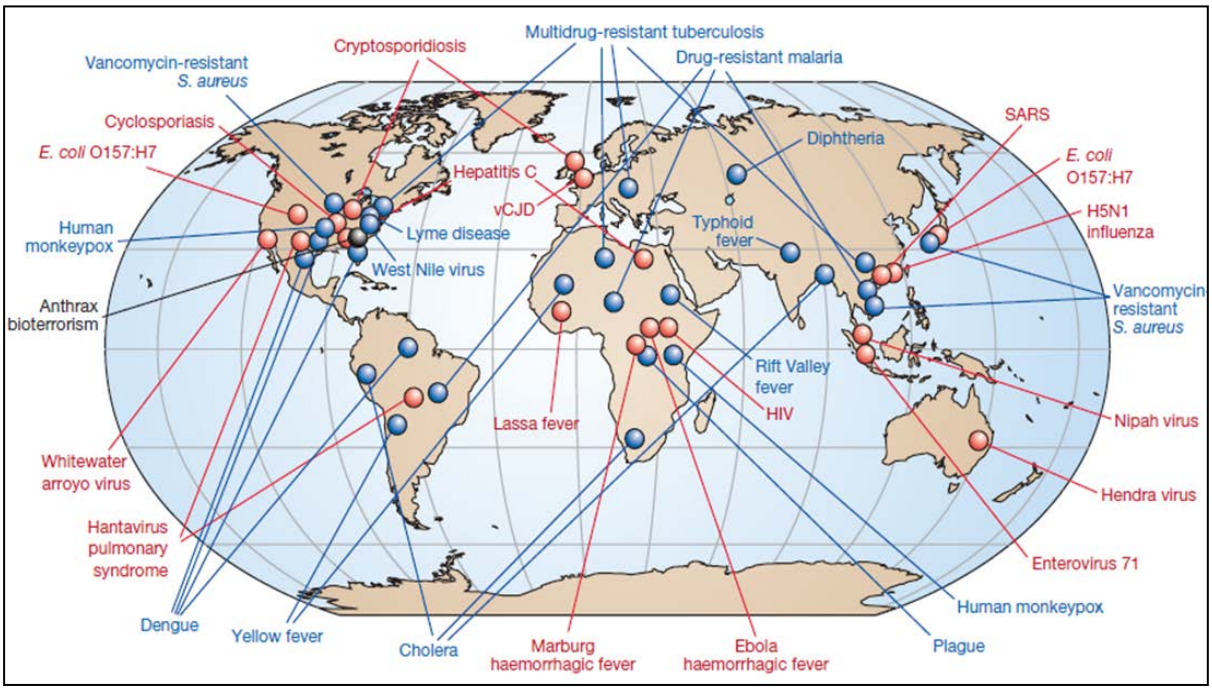

Figure 2. Exemples mondiaux de maladies émergentes ou ré-émergentes (Morens et al., 2004).

1.2. Différentes étapes dans l'émergence et facteurs impliqués

Morse et al. (2012), en s'appuyant sur le modèle de Daszak et al. (2000), ont décomposé le processus pouvant conduire à une zoonose chez l'homme en 3 étapes (**Figure 3**). La première étape, pré-émergence, correspond aux perturbations écologique, sociale, socio-économique ou encore génétique qui modifient la dynamique de transmission d'un agent infectieux à l'intérieur de son réservoir, le poussant à se multiplier et augmenter sa densité, à diffuser dans de nouvelles régions, et à de nouveaux hôtes non humains, le plus souvent de la faune domestique (épizooties). Il s'agit de l'étape à laquelle nous nous sommes particulièrement intéressés aux cours de ces travaux. La seconde étape correspond au phénomène de saut d'espèce du pathogène depuis les réservoirs de la faune sauvage ou domestique vers les populations humaines, c'est l'étape d'émergence. Enfin, la dernière étape correspond à la transmission du pathogène entre populations humaines, à son expansion au niveau mondial par divers moyen de transport, c'est l'étape de pandémie.

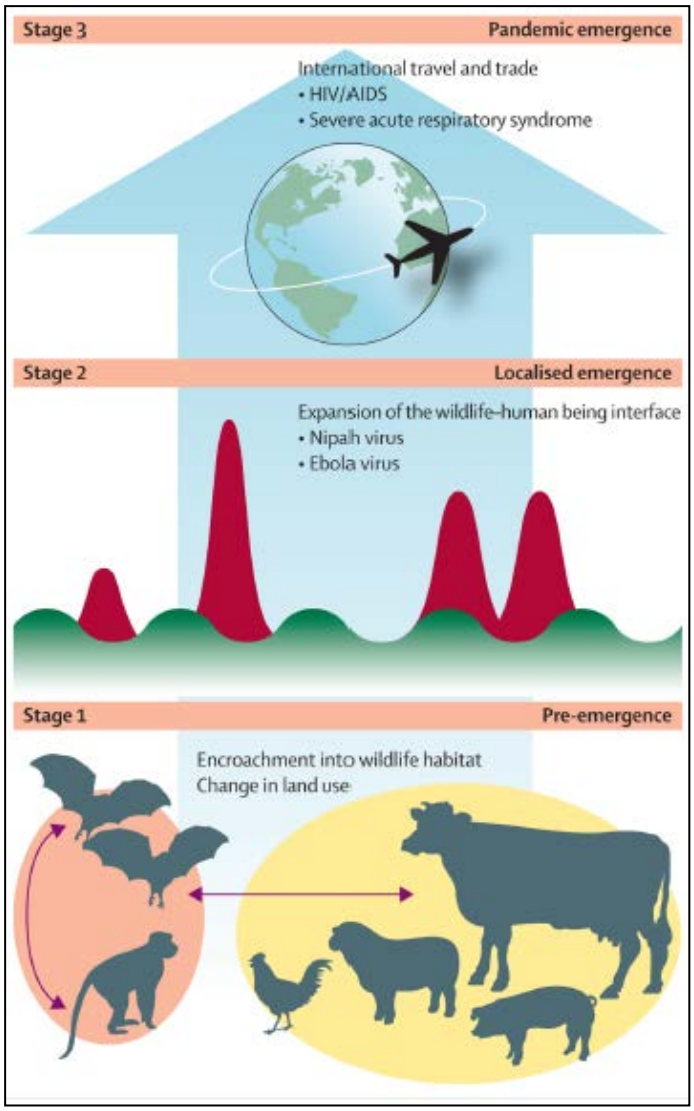

Figure 3. Différentes étapes d'émergences d'agents zoonotiques (Morse et al., 2012).

De nombreuses études ont ainsi décrit des facteurs écologiques et des processus évolutifs qui seraient potentiellement impliqués dans le phénomène de sauts d'espèce. L'un des facteurs capitale serait le changement dans l'environnement ou dans le comportement des hôtes réservoirs qui pourrait permettre que de nouvelles niches écologiques soient exploitées par des agents infectieux hébergés *via* des sauts entre espèces voisines compatibles et/ou permissives. Ces changements provoqueraient un déséquilibre entre un hôte et un agent pathogène évoluant dans un écosystème donné (Schrag & Wiener 1995 ; Daszak et al., 2000 ; Morand et al., 2006 ; Plowright et al., 2015) (**Figure 4**).

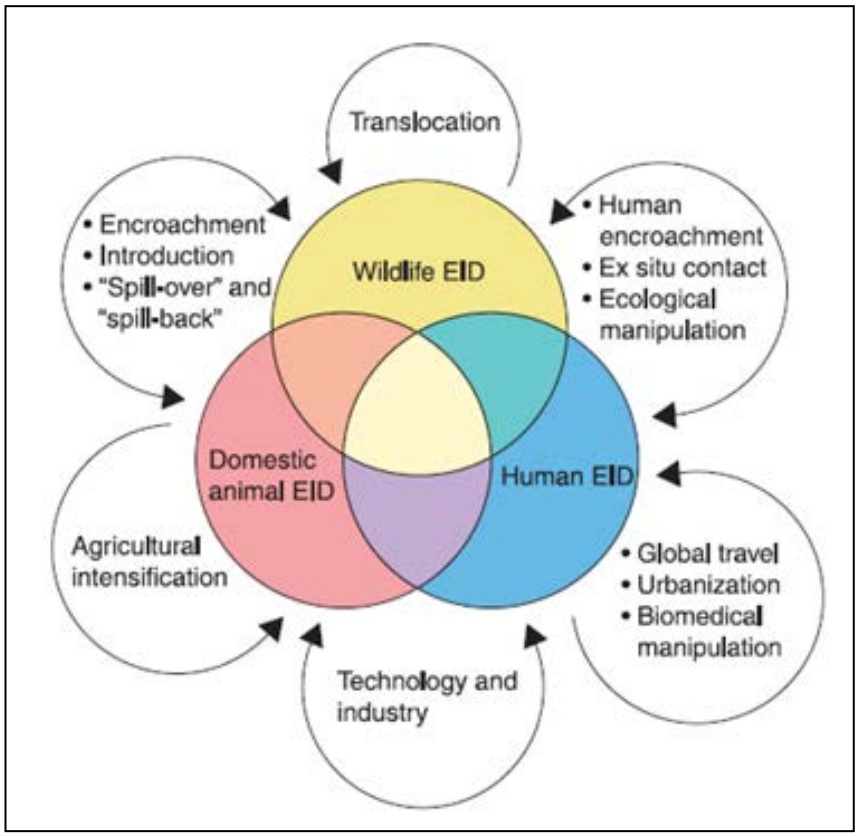

Figure 4. Continuum entre populations d'hôtes-agents zoonotiques et facteurs d'émergences (Daszak et al., 2000).

1.2.1. Facteurs anthropogéniques

Les modifications des écosystèmes, les comportements humains, les activités socioéconomiques et les bouleversements démographiques (développement économique, déplacements massifs de populations, échanges internationaux, intensification des élevages), les résistances aux antibiotiques ou encore des catastrophes naturelles sont autant de facteurs pouvant conduire à ce déséquilibre (Daszak et al., 2000 ; Woolhouse et al., 2005 ; Food and Agriculture Organisation of the United Nations, 2011). C'est typiquement le cas des évènements de cohabitation générés par la levée du filtre de rencontre entre espèces allopatriques, devenues alors sympatriques au sein des mêmes écosystèmes.

L'épidémie du virus Nipah illustre clairement l'influence des comportements humains et des perturbations environnementales sur l'apparition de maladies émergentes infectieuses. Le virus *Nipah* (Famille : *Paramyxoviridae*, Genre : *Henipavirus*) a été décrit pour la première fois suite à une épidémie majeure chez les porcs et les humains dans la péninsule malaise entre septembre 1998 et avril 1999. Plus d'un million de porcs ont été abattus pour contenir l'épidémie. Au moins 265 cas humains et 105 décès ont été signalés (Chua et al., 2000). Le contact direct avec des porcs infectés a été identifié comme le principal mode de contamination chez l'homme. Les chauves-souris du genre *Pteropus* ont été reconnues comme hôtes naturels du virus (Johara et al., 2001). On sait que c'est l'entassement des cochons dans des enclos situés à proximité des colonies de chauves-souris frugivores du genre *Pteropus* porteuses qui en est la cause majeure. Cette proximité entre les deux espèces était elle-même la conséquence de la perte par les chauves-souris de leur habitat naturel et des ressources alimentaires frugales détruits par la déforestation malaise et indonésienne en faveur des programmes de plantation intensive de palmiers à huile. Il en a résulté une migration des chauves-souris vers les enclos de porcs à la recherche de nouvelles sources de nourriture (**Figure 5**). Il a été démontré que les gouttelettes d'urines émises en vol par ces chauves-souris contiennent des traces du virus qui ont pu constituer des sources de contamination des porcs. Le virus est secondairement transmis par aérosol, directement entre porcs et des porcs infectés vers l'homme (Chua et al., 2000).

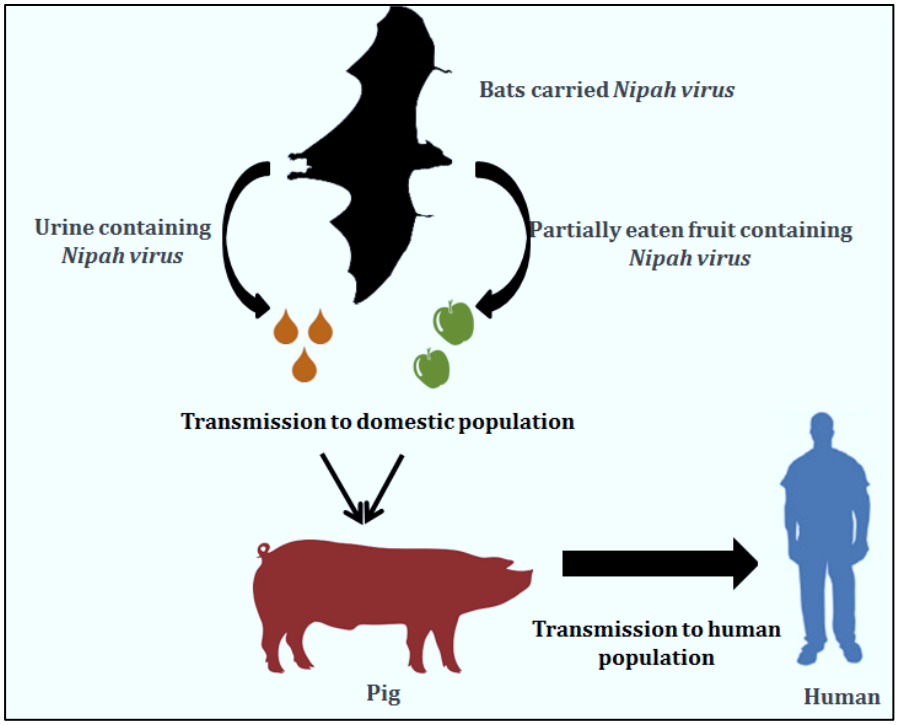

Figure 5. Transmission du virus *Nipah* chez l'homme par les chiroptères *via* la faune domestique.

1.2.2. Facteurs écologiques

A l'échelle de la faune, la richesse et la diversité d'hôtes et d'agents zoonotiques au sein d'une communauté ont été décrites comme des facteurs importants dans le maintien et la transmission d'agents zoonotiques (Ostfeld, 2000 ; Woolhouse et al., 2005 ; Keusch et al., 2009). Keesing et al. (2010) ont par ailleurs souligné que les changements en richesse spécifique associés aux modifications que les facteurs essentiellement anthropogéniques imposent à la biodiversité peuvent impacter les évènements d'émergences infectieuses par un effet de dilution ou un effet d'amplification. L'analyse de tels facteurs est déterminante dans la compréhension de la transmission d'agents zoonotiques. Se concentrer ainsi non pas sur une seule espèce, mais toute une communauté d'hôtes permet de comprendre le rôle de chaque espèce dans la séquence des interactions hôtes-pathogènes (Morand et al., 2014). Par ailleurs, de nombreuses études ont montré l'importance des facteurs biotiques et abiotiques dans la transmission d'agents zoonotiques. O'Shea et al. ont décrit par exemple l'importance de l'âge, du sexe, de la saison et du climat dans la circulation du virus de la rage chez une communauté de chauves-souris au Colorado (O'Shea et al., 2014). Streicker et al. ont rapporté le rôle du climat tropical et subtropical favorisant la transmission inter-espèces du virus de la rage chez les chauves-souris (Streicker et al., 2012). George et al. ont montré que l'état de torpeur pendant les périodes hivernales diminuait l'activité virale chez les chauves-souris (Georges et al., 2011).

1.2.3. Facteurs génétiques

Plus loin dans leur étude, Morse et al. (2012) identifient un ensemble de facteurs génétiques qui apparaissent liés au phénomène d'émergence, parmi lesquelles on peut citer : i) la parenté phylogénétique avec l'hôte primaire hébergeant un agent zoonotique qui favoriserait le saut d'espèce d'un virus (Streicker et al., 2010), comme c'est le cas du virus de l'immunodéficience humaine (VIH) des chimpanzés, ii) la parenté phylogénétique entre agents pathogènes de la faune sauvage et ceux infectant les populations humaines (Pulliam, 2008) iii) le large spectre d'hôte ainsi qu'une certaine plasticité génétique virale (mutation, recombinaison, réassortiment) génèrent une diversité populationnelle (Woolhouse et al., 2001 ;Woolhouse et al., 2005 ; Parrish et al., 2008), iv) la présence de récepteurs cellulaires « ubiquitaires » qui favoriserait un large tropisme tissulaire ou un changement d'hôte (Pepin et al., 2010 ; Woolhouse et al., 2012), et enfin, v) les relations co-évolutives entre un hôte et son microbiote (Poulin & Morand, 2004) sont des déterminants importants de l'émergence d'un agent zoonotique (Cui et al., 2007 ; Olival et al., 2014), un parasite exprimant un fort pattern de co-évolution avec son hôte ne fera que très peu de « *host-switch* », alors qu'un parasite co-évoluant faiblement avec son hôte sera plus à même de sauter la barrière d'espèce. Ces relations passent essentiellement par deux processus évolutifs à savoir, la macro-évolution et la micro-évolution.

1.2.3.1. Macro-évolution

Les processus macro-évolutifs sont des mécanismes se déroulant généralement sur une échelle de temps longue et vont être en partie à l'origine de la diversité génétique d'un parasite. Ils regroupent deux mécanismes principaux: la "co-spéciation" et le saut d'espèce ou "*host-switch*" (Page, 2003). La co-spéciation correspond à une diversification de l'hôte, entraînant à sa suite une co-divergence du parasite qui lui est fortement adapté (**Figure 6**). A l'inverse, le saut d'un parasite correspond à un passage depuis l'hôte ancestral vers un nouvel hôte, passage suivi d'une spécialisation générant une nouvelle combinaison hôte-microorganisme (Agosta et al., 2010 ; de Vienne et al., 2013). Certaines études qui plaidaient en faveur d'un rôle prépondérant de la co-spéciation dans les processus macro-évolutifs doivent être interpréter avec précaution du fait d'une probable surestimation due à l'utilisation d'outils inadaptés en phylogénie (de Vienne et al., 2013). C'est le cas du modèle hantavirus

souvent cité comme prototypique de l'association des virus à leurs hôtes respectifs présentant une parfaite congruence entre les arbres phylogénétiques des hôtes et des différents lignages viraux (Hughes & Friedman, 2000; Jackson & Charleston 2004; Ramsden et al., 2009). Cependant, il a par la suite été démontré que le moteur réel de leur évolution dépendait plus des multiples évènements de transmission par "*host-switch*", que d'un mécanisme de co-spéciation des hôtes (Ramsden et al., 2009; Lin et al., 2012 ; Guo et al., 2013). D'autres événements évolutifs, tel que la duplication, correspondant à la spéciation indépendante du parasite dans son hôte ainsi que le « *sorting* » correspondant le plus généralement à (i) l'absence de colonisation d'un parasite dans les différentes lignées de l'hôte (« *Missing the boat* ») ou à (ii) l'extinction de la lignée parasitaire après la co-spéciation (« *extinction* ») (iii) ou à l'absence de spéciation du parasite lors de la spéciation de l'hôte (« *failure to speciate* ») (**Figure 6**) (Page, 2003).

1.2.3.2. Micro-évolution

Les processus micro-évolutifs agissent sur des échelles de temps beaucoup plus courtes, et regroupent les interactions hôte-agent infectieux que l'on décrit généralement comme celles qui permettent, suite à un « *host-switch* », la meilleure adaptation de l'agent infectieux à son hôte. Il s'agit d'une "coévolution" *sensu stricto* (de Vienne et al., 2013), c'est-à-dire issue de la pression de sélection réciproque entre l'hôte et l'agent infectieux: échappement d'un agent infectieux, fixation des mutations, recombinaisons et réassortiments, etc. (Domingo & Holland, 1997). Cette forte dynamique évolutive entraîne l'apparition d'une grande diversité de variants génétiquement proches et qui vont constituer une population virale particulière appelée « quasi-espèce » (Domingo et al., 1999).

Les processus qui mettent en jeu une micro-évolution ne sont nullement exclusifs de ceux qui dirigent la macro-évolution, et peuvent être sujets à la coopération. Par exemple, après transmission d'un parasite à un nouvel hôte par *host-switch*, il est nécessaire pour ne pas connaitre l'extinction, que le pathogène s'adapte au mieux à son nouvel environnement, son nouvel hôte via ces mécanismes évolutifs.

Il est donc intéressant et important d'investiguer ainsi à la fois les facteurs écologiques et les processus macro/micro évolutifs qui influeraient sur la diversité et la transmission d'agents zoonotiques inter et intra populationnelle. Au cours de nos travaux nous nous sommes

intéressés aux animaux réservoirs dont certains hébergent des agents pathogènes présentant des caractéristiques décrites ci-dessus.

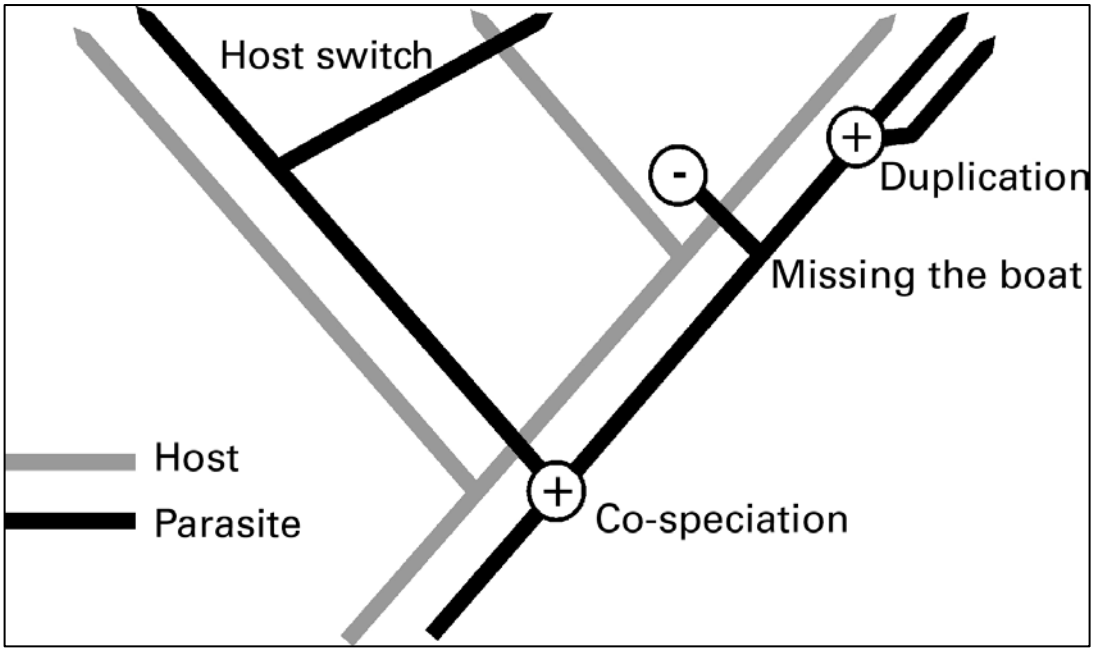

Figure 6. Mécanismes évolutifs hôte-parasites (issue de Michel Widmann, ENS Lyon, adapté de Paterson, 1997 et Taraschewski, 2006).

Chapitre 2. Micromammifères sauvages un réservoir d'agents zoonotiques

2.1. Généralités

La faune sauvage joue un rôle clé dans l'émergence des agents pathogènes dans les populations d'animaux domestiques et les populations humaines en servant de «réservoir enzootique" source d'agents infectieux pour la plupart antérieurement inconnus qui peuvent s'adapter, se répliquer et émerger dans de nouveaux hôtes (Morse et al., 1993). A cet égard l'exploration des agents infectieux dans la faune sauvage revêt un grand intérêt afin d'anticiper leur émergence potentielle et le déclenchement des maladies infectieuses nouvelles. Durant mes travaux de thèse, je me suis intéressé à deux réservoirs majeurs d'agents zoonotiques particuliers, les chauves-souris et les petits mammifères terrestres (Woolhouse et al., 2012).

2.2. Les chauves-souris

2.2.1. Caractéristiques

Les chauves-souris (Order Chiroptera) représentent un groupe très diversifié de mammifères que l'on retrouve pratiquement dans l'ensemble du globe à l'exception des régions polaires et quelques îles océaniques. Le terme *chiroptère* est dérivé du grec *kheir* (ἡ χείρ) et désigne la main et le terme *ptéron* (*τὸ πτερόν*) désignant l'aile. Elles représentent l'un des plus grands ordres de mammifères au monde avec plus de 1200 espèces (UICN pour Union Internationale pour la Conservation de la Nature, 2010), soit 20% des mammifères terrestres, et occupent le second rang après les rongeurs en termes de nombre de genres et d'espèces (Koopman, 1993). Elles sont classées en deux ordres : les Yangochiroptera (anciennement appelées Microchiroptéres) et Yinpterochiroptera (anciennement appelées Megachiroptéres) (Koopman, 1993). L'ordre des Yangochiroptera est constitué de deux sous-ordres et contient le plus grand nombre d'espèces, près de 1000 (UICN, 2010), et a une plus grande diversité écologique. La plupart des familles de cet ordre sont insectivores, bien que certaines familles aient un comportement polyphage.

L'ordre Yinpterochiroptera comprend plus de 200 espèces (UICN, 2010) dont la taille est en moyenne bien supérieure à celle des espèces de l'ordre Yangochiroptera (Mickleburgh & Hutson, 1992). Elles se nourrissent principalement de fruits et de nectar. Les chauves-souris frugivores sont surtout retrouvées dans les régions sub-tropicales et tropicales de l'Ancien Monde ; en Méditerranée orientale et la péninsule arabique, en Afrique et en Asie, en Australie et les îles du Pacifique et de l'Océan Indien (Rainey & Pierson, 1992). Bien que l'analyse des fossiles permettant de retracer l'évolution des chauves-souris reste incomplète (Jones et al., 2002), une datation a permis d'estimer l'origine des chiroptères à la période éocène, il y a environ 52 à 50 millions d'années coïncidant avec l'augmentation significative de la température mondiale (Teeling et al., 2005). Généralement longévives, elles sont dotées de grandes capacités de dispersion sur de grandes distances. Elles forment des colonies densément peuplées (**Figure 7**) et présentent des comportements sociaux qui structurent leurs populations. Elles sont capables de rentrer en hibernation et possèdent un système

immunitaire qui leur confère une tolérance remarquable vis-à-vis d'agents infectieux (Calisher et al., 2006 ; Turmelle & Olival, 2009).

Le rôle des chauves-souris dans la transmission de pathogènes et l'émergence des maladies infectieuses d'origine virale est maintenant bien établi dans de nombreuses régions du monde (Sulkin & Allen, 1974 ; Wong et al., 2007 ; Wang, 2009). Des études récentes ont par ailleurs montré que les chauves-souris sont des hôtes depuis des temps très anciens de lyssavirus (Badrane & Tordo, 2001 ; Streicker et al., 2010 ; Streicker et al 2012 ; Banyard et al., 2014) et les henipavirus (Calisher et al., 2006 ; Drexler et al., 2012), suggérant une longue co-spéciation entre ces virus et les chauves-souris.

Figure 7. Colonie de *Mormopterus francoismoutoui* (insectivores) dans la grotte de Trois-Bassins à La Réunion (Crédits photo : Erwan Lagadec).

2.2.2. Réservoirs d'agents pathogènes

Les chauves-souris sont reconnues comme les réservoirs les plus importants d'agents zoonotiques (Calisher et al., 2006) sans doute les plus anciens (Vidgen et al., 2015). Calisher et al. (2006) ont fait état de plus de 66 virus isolés ou détectés à partir de tissus de 74 espèces de chauves-souris (**Tableau 1**). Certains de ces virus sont connus pour causer des maladies humaines et animales. Les chauves-souris possèdent certaines caractéristiques qui peuvent maximiser leur efficacité en tant que réservoir et pourvoyeur de virus (Calisher et al., 2006). Certaines espèces de Yinpterochiroptera souvent perchées dans les arbres et fortement agrégés, forment des colonies considérables (**Figure 8**). L'occupation de ce genre de site peut être saisonnière (par exemple, pendant les périodes d'hibernation ou de maternité) ou pérenne, tout au long de l'année (Kunz & Pierson, 1994). Selon les espèces, la saison, et l'emplacement du site, la taille des colonies va de quelques spécimens à de milliers d'individus. C'est ainsi que les chauves-souris sont connues pour former les plus grands rassemblements comparés à tous les autres mammifères.

Cette propriété est particulièrement remarquable chez les colonies d'insectivores tels les Molossidae et les Vespertilionidae qui ont les plus grands comportements grégaires (McCracken & Gustin, 1991) (**Figure 7**). Plus d'un tiers des espèces de Yinpterochiroptera forme des colonies (Marshall, 1983 ; Pierson & Rainey, 1992). Des études ont montré que le contact étroit entre chauves-souris ayant ce mode de vie serait favorable la transmission d'agents infectieux (Luis et al., 2015).

Tableau 1. Exemple de virus isolés de chauves-souris (Calisher et al., 2006).

Virus	Bat species (common name)[a]
Family *Rhabdoviridae*, genus *Lyssavirus*	
Rabies virus	Numerous bat species, essentially worldwide
Lagos bat virus	*Eidolon helvum* (African straw-colored fruit bat), *Micropteropus pusillus* (Peters' lesser epauletted fruit bat), *Epomops dobsonii* (Dobson's epauletted fruit bat), *Nycteris gambiensis* (Gambian slit-faced bat), *Epomophorus wahlbergi* (Wahlberg's epauletted fruit bat)
Duvenhage virus	*Miniopterus* sp., *Nyctalus noctula* (noctule), *Vespertilio murinus* (particolored bat), *Nycteris thebaica* (Egyptian slit-faced bat)
Australian bat lyssavirus	Megachiroptera (multiple *Pteropus* spp.), Microchiroptera sp. from Australia, *Saccolaimus flaviventris* (yellow-bellied pouched bat)
European bat lyssavirus 1	*Eptesicus serotinus* (common serotine), *Rousettus aegyptiacus* (Egyptian rousette)
European bat lyssavirus 2	*Myotis myotis* (mouse-eared myotis), *Myotis dasycneme* (pond myotis), *Myotis nattereri* (Natterer's myotis), *Miniopterus schreibersii* (Schreibers' long-fingered bat), *Rhinolophus ferrumequinum* (greater horseshoe bat), *Myotis daubentonii* (Daubenton's myotis)
Aravan virus	*Myotis blythii* (lesser mouse-eared myotis)
Khujand virus	*Myotis mystacinus* (whiskered myotis)
Irkut virus	*Murina leucogaster* (greater tube-nosed bat)
West Caucasian bat virus	*Miniopterus schreibersii* (Schreibers' long-fingered bat)
Family *Rhabdoviridae*, genus unassigned	
Gossas virus	*Tadarida* sp.
Kern Canyon virus	*Myotis yumanensis* (Yuma myotis)
Mount Elgon bat virus	*Rhinolophus eloquens* (eloquent horseshoe bat)
Oita 296 virus	*Rhinolophus cornutus* (little Japanese horseshoe bat)
Family *Orthomyxoviridae*, genus *Influenzavirus A*, influenza A virus	*Nyctalus noctula* (noctule)
Family *Paramyxoviridae*, genus *Rubulavirus*	
Mapuera virus	*Sturnira lilium* (yellow epauletted bat)
Menangle virus	*Pteropus poliocephalus* (gray-headed flying fox)
Tioman virus	*Pteropus hypomelanus* (variable flying fox)
Family *Paramyxoviridae*, genus undetermined, a parainfluenzavirus	*Rousettus leschenaultia* (Leschenault's rousette)
Family *Coronaviridae*, SARS coronavirus	*Rhinolophus sinicus* (Chinese horseshoe bat), *Rhinolophus pearsonii* (Pearson's horseshoe bat), *Rhinolophus macrotis* (big-eared horseshoe bat), *Rhinolophus ferrumequinum* (greater horseshoe bat)

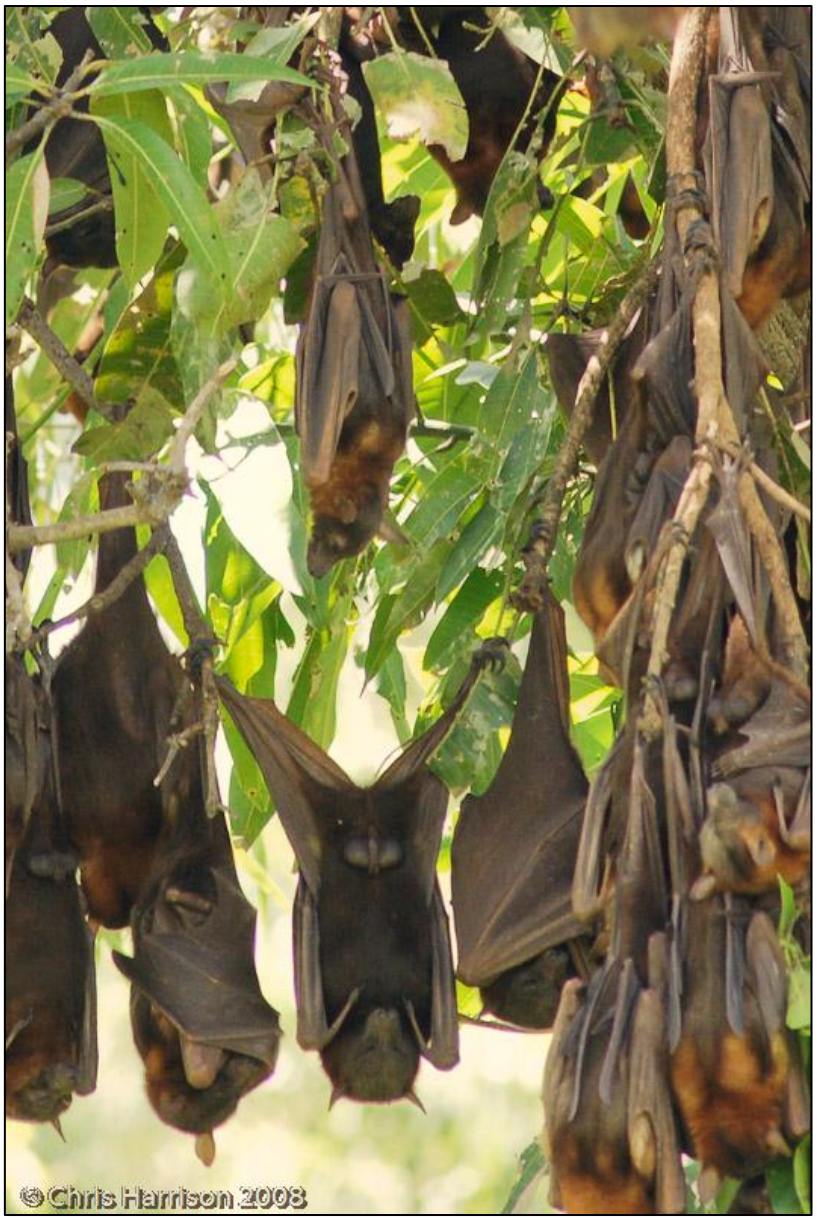

Figure 8. Colonie de *Pteropus scapularis* (frugivores) perchés sur un arbre (Crédits photo : Harrison Chris).

La durée de vie de nombreuses espèces de petites chauves-souris de l'ordre des Yangochiroptera, particulièrement celles vivant dans des régions tempérées, dépasse 25 ans, avec la plus grande longévité estimée à 35 ans, pour les frugivores. Cette longévité importante par rapport à leur taille relativement petite, est un trait de vie considéré comme favorisant l'infection et associée à des infections persistantes pourrait expliquer en partie le rôle de transmetteur efficace d'agents infectieux (Calisher et al., 2006).

Le régime alimentaire des chauves-souris est très diversifié allant du régime généraliste à très spécialisé. Les insectivores se nourrissent d'un large éventail d'arthropodes, notamment d'insectes mais aussi de mites, d'araignées et de petits crustacés (Kunz & Pierson, 1994). Certaines espèces sont frugivores se nourrissant de fruits et de nectars (von Helversen, 1993) alors que d'autres sont carnivores. Une dernière catégorie de chauves-souris est sanguivore, se nourrissant principalement de sang de mammifères et d'oiseaux (Kunz & Pierson, 1994). Les chauves-souris sanguivores revêtent une grande importance d'un point de vue santé humaine et vétérinaire dans les pays du Nouveaux Monde où la transmission d'agents zoonotiques se produit lors des morsures, notamment pour le virus de la rage (Constantine, 1988).

Enfin, le comportement migratoire de certaines espèces de chauves-souris a aussi été incriminé dans la transmission d'agents zoonotiques. Certaines espèces de chauves-souris sont capables de voler sur de longues distances particulièrement lors des migrations saisonnières (Griffin, 1970). Les espèces de Yangochiroptera voyagent souvent entre 10 à 15 km mais peuvent parcourir de plus longues distances dans leur quête de nourriture (Kunz & Pierson, 1994). Certaines espèces de Yinpterochiroptera sont également connues pour parcourir des distances au-delà de 80 km pour se nourrir (Epstein et al., 2009). Les Yangochiroptera dans les régions tempérées du nord, migrent en période hivernale vers le sud pour bénéficier d'un climat moins extrême (Strelkov et al., 1969).

2.3. Les petits mammifères terrestres

2.3.1. Caractéristiques

Les rongeurs (ordre : Rodentia) représentent l'ordre le plus abondant et le plus diversifié des mammifères et comporte environ 43% du nombre total d'espèces de mammifères vivant dans le monde (Huchon et al 2002; Wilson & Reeder 2005). Le genre *Rattus* (sous famille Murinae, famille des Muridae, super famille *Muroidae*) est le plus important avec plus de 60 espèces inventoriées (Aplin et al., 2003). *Rattus norvegicus* (rats bruns) et *Rattus rattus* (rats noirs) sont parmi les deux espèces les plus répandues et sont communément appelées rongeurs de l'Ancien Monde (Wilson & Reeder, 2005). *Rattus norvegicus* est connu pour être originaire du nord de la Chine (Nowak & Walker, 1991) alors que *R. rattus* proviendrait de l'Inde et d'Asie du Sud (Bonnefoy et al., 2008 ; Nagorsen, 2005). Les premiers membres sont apparus il y a environ trois millions d'années, dans le Pliocène tardif et se sont dispersés rapidement à travers l'Asie du Sud (Aplin et al., 2003). Ils ont depuis réussi à coloniser tous types d'habitats et se retrouvent sur tous les continents sauf en Antarctique (Lund, 1994). Leur expansion mondiale a été favorisée par les moyens de transport en particulier le trafic maritime (Bonnefoy et al., 2008 ; Nagorsen, 2005). *Rattus norvegicus* est plus fortement associés aux écosystèmes urbains (Yahner, 2001) tandis que *R. rattus* sera communément retrouvé dans les zones rurales. Ces deux espèces de rongeurs sont connues pour être des ravageurs de cultures causant des destructions importantes de récoltes, notamment en Asie du Sud et du Sud-est (Aplin et al., 2003).

2.3.2 Réservoirs d'agents pathogènes et perte de la biodiversité

Les petits mammifères terrestres, notamment les rats tels que *Rattus norvegicus* et *R rattus* sont eux aussi identifiés comme source d'un certain nombre d'agents pathogènes responsables de morbidité et mortalité importantes dans les zones urbaines de différentes régions du monde. En terme de santé publique, *R rattus* et *R norvegicus* sont les deux rongeurs les plus incriminés dans la dissémination d'agents pathogènes responsables de bactérioses (la leptospirose due à *Leptospira*, la peste causée par *Yersinia* et des Rickettsioses comme le typhus murin dues à *Rickettsia*) et de viroses (syndrome pulmonaire à hantavirus) responsables de morbidité et mortalité humaines importantes à travers le monde (Meerburg et al., 2009).

Meerburg et al. (Meerburg et al., 2009) ont fait état de plusieurs agents pathogènes isolés de rongeurs (**Tableau 2**). Parmi eux, les infections à hantavirus du genre *Hantavirus* (Famille : *Bunyaviridae*) sont les plus connues avec le syndrome pulmonaire à hantavirus. Les hantavirus sont aussi retrouvés chez d'autres petits mammifères terrestres, notamment ceux appartenant à la famille des Cricetidae Murnidae, Soricidae et Talpidae (Guo et al., 2014).

Tableau 2. Aperçu des différentes maladies et agents pathogènes retrouvés chez les rongeurs (Meerburg et al., 2009).

Disease	Agent	Carrier/Reservoir	Population at-risk	Chance	Severity	
					Human Health	Economy
Hantavirus Pulmonary Syndrome	Virus, Bunyaviridae	Carrier	2	1	3	1
Hemorrhagic Fever with renal syndrome (+ other hemorrhagic fevers)	Virus, Bunyaviridae	Carrier	2	2	2	2
Nephropathia epidemica	Virus, Bunyaviridae	Carrier	1	1	1	1
Crimean-Congo hemorrhagic fever	Virus, Bunyaviridae	Reservoir	1	1	3	1
Borna disease	Virus, Bornaviridae	Reservoir	1	1	1	2
Omsk hemorrhagic fever	Virus, Flaviviridae	Reservoir	1	1	1	1
Kyasanur Forest Disease	Virus, Flaviviridae	Reservoir	1	1	1	1
Apoi Virus Disease	Virus, Flaviviridae	Unknown	Unknown	Unknown	Unknown	Unknown
Tick-borne encephalitis	Virus, Flaviviridae	Reservoir	2	1	3	1
Powassan encephalitis	Virus, Flaviviridae	Reservoir	1	1	1	1
Lymphocytic	Virus, Arenaviridae	Reservoir	1	1	1	1

Reservoir: rodents harbor disease-causing organisms and thus serve as potential sources of disease outbreaks, but always via a vector (tick, sand-fly etc.)
Carrier: rodent that shows no or limited symptoms of a disease but harbors the disease-causing agent and is capable of passing it directly onto humans
Population at-risk: focal = 1, regional = 2, more than 2 continents = 3
Chance: chance of contracting the disease (all pathways, not only via rodents): small chance = 1, moderate chance = 2, high chance = 3
Human health: Mortality without treatment <5%=1, 5 to 10% = 2, >10% = 3. No mortality = 0.
Economy: losses in terms of morbidity combined with other losses (e.g. in animal productivity): small losses = 1, moderate losses = 2, huge losses = 3.

La caractéristique principale des rongeurs, aggravante en terme de risque infectieux est le contact étroit qu'ils établissent avec les populations humaines. La transmission de pathogènes peut alors se produire soit de façon directe, par morsure la plupart du temps, soit de façon indirecte, par contamination des produits alimentaires et du milieu par les fèces ou les urines, par formation d'aérosols contagieux ou médiée par des vecteurs arthropodes ectoparasites (tiques, acariens, puces) (Meerburg et al., 2009). La grande taille des populations, résultant de leur taux de reproduction extrêmement élevé, augmente le risque de contact et de transmission d'agents pathogènes vers les populations humaines. Ces deux espèces de rongeurs sont connues pour être des ravageurs de cultures causant des destructions importantes de récoltes, notamment en Asie du Sud et du Sud-est (Aplin et al., 2003). *Rattus rattus* et *R. norvegicus* sont à l'origine d'invasion biologique pouvant avoir des effets dévastateurs sur les écosystèmes naturels en déplaçant et réduisant les populations indigènes, comme les oiseaux, les reptiles ou encore les végétaux (Nowak & Walker, 1991). Cette invasion par des populations de rongeurs peut entraîner l'extinction d'autres espèces animales en particulier endémiques (Clavero et al., 2005) avec une réduction de la biodiversité surtout en territoire insulaire. L'appauvrissement spécifique peut favoriser la transmission épidémique des agents infectieux quand les espèces tampons viennent à disparaître au profit d'une espèce invasive vectrice (Keesing et al., 2006).

Chapitre 3. Caractéristiques de la région du Sud-ouest de l'Océan Indien (SOOI)

3.1. Généralités

Les îles tropicales pour lesquelles nous avons axé notre étude sont Madagascar, et les archipels des Comores (Mayotte et Anjouan), des Seychelles (Mahé) et des Mascareignes (La Réunion et Maurice) (**Figure 9**). Cet ensemble insulaire est remarquable par la diversité des îles qui le composent: diversité de leur origine géologique, de leur âge, des origines ethniques des populations humaines qui le peuplent, disparités de leurs développements économiques, et des voies d'échanges internationales et commerciales. Parmi les îles du SOOI, Madagascar a plusieurs caractéristiques écologiques exceptionnelles : Six types de végétations, quatre régions bioclimatiques et une échelle altitudinale allant de 0 à 2875 m (Goodman & Raherilalao, 2013).

Typiquement, la faune vertébrée des îles du SOOI proviendrait de l'Afrique ou encore de l'Australasie (Peake et al., 1971). Ces îles font partie des 34 régions de notre planète considérées par l'UICN comme « hotspots » de la biodiversité (Myers et al., 2000).

Nous avons porté notre intérêt sur les chauves-souris et les petits mammifères terrestres de ces îles. La plus grande richesse et le plus grand taux d'endémisme pour ces deux groupes d'animaux sont observés sur la grande île de Madagascar. On y dénombre aujourd'hui le plus d'espèces de petits mammifères non volants, un total de 45 espèces de chauves-souris (3 frugivores et 42 insectivores), dont 36 sont endémiques à l'île et plus de 64 espèces de petits mammifères terrestres (Goodman, 2011 ; Soarimalala & Goodman, 2013).

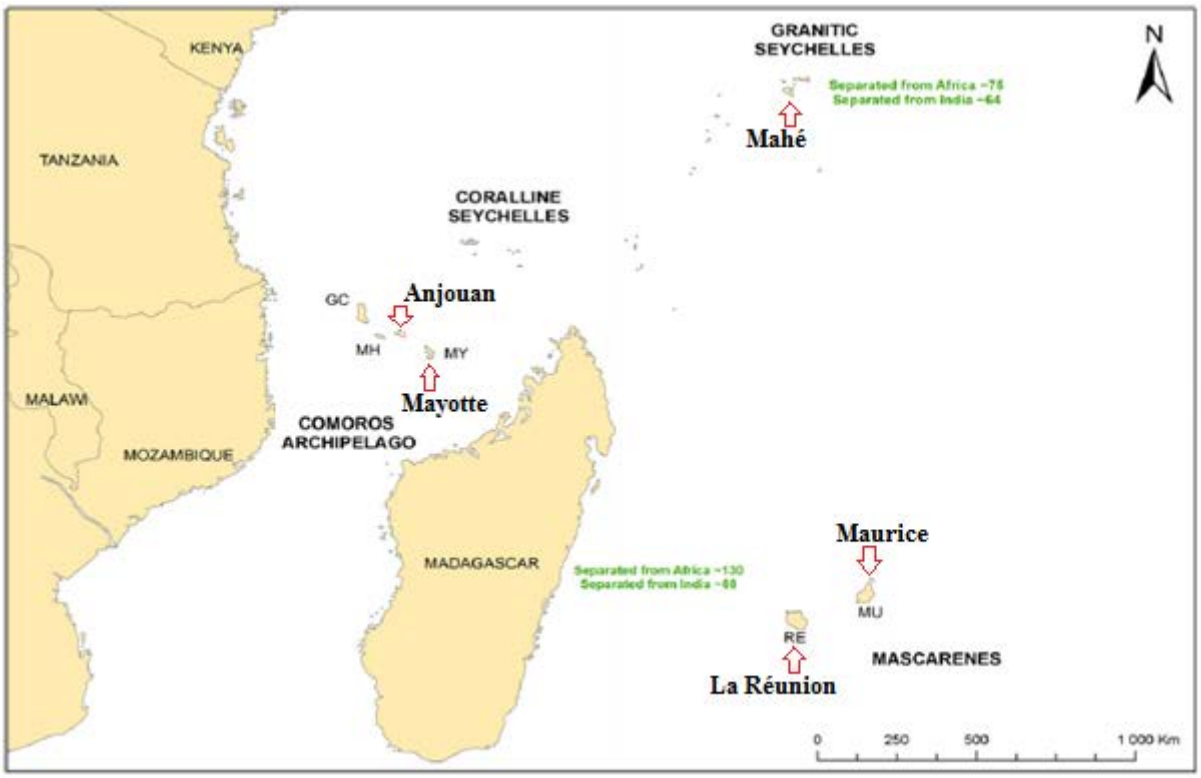

Figure 9. Les îles du Sud-ouest de l'Océan Indien (adapté de Tortosa et al., 2012).

3.2. Les chauves-souris

Dans un récent atlas, Goodman & Ramasindrazana, (2013) ont décrit les familles et espèces de chauves-souris connues à ce jour pour être retrouvées à Madagascar. Par ailleurs, ils ont inscrit différentes caractéristiques écologiques intéressantes et importantes. La plupart des sites connus pour héberger des chauves-souris sont constitués de communautés d'espèces parfois de plusieurs familles différentes vivant en sympatrie, particulièrement chez les chauves-souris de la famille de Miniopteridae qui, avec les Vespertilionidae, représente les deux plus grandes familles de chauves-souris de Madagascar. Les chauves-souris de ces îles vivent majoritairement dans les grottes (cavernicoles) ou dans les forêts ou feuillage (forestière), mais certaines espèces sont rencontrées proche des habitations humaines (synantropique) notamment chez les chauves-souris de la famille des Molossidae. Plusieurs espèces peuvent être retrouvées sur une étendue géographique importante allant du Sud au Nord de l'île (Goodman, 2011). Ces modes de vie contrastés sont intéressants, d'une part, pour analyser la transmission inter-espèces de pathogènes, notamment viraux, et d'autre part, déterminer le risque de transmission infectieuse des chauves-souris vers les populations humaines. Le **tableau 3** décrit les caractéristiques connues des chauves-souris retrouvées à Madagascar qui ont fait l'objet de notre étude (Goodman, 2011). Certaines espèces ont été récemment décrites (Goodman et al., 2012a ; Goodman et al., 2012b ; Goodman & Ramasindrazana, 2015 ; Goodman et al .2015). Par ailleurs, la revue d'O'Brien et al. (2011) décrit les différentes espèces de chauves-souris présentes sur les îles de la zone SOOI. Certaines espèces comme *Taphozous mauritianus* sont retrouvées en commun sur de nombreuses îles.

Le genre *Mormopterus*, dont les espèces sœurs *Mormopterus jugularis*, *Mormopterus francoismoutoui* et *Mormopterus acetabulosus,* est retrouvé sur Madagascar, La Réunion et Maurice, respectivement (Goodman et al., 2008).

Le **tableau 4** décrit quant à lui les différentes chauves-souris des autres îles de la zone du SOOI étudiées (O'Brien, 2011).

Tableau 3. Caractéristiques et particularités des chauves-souris collectées à Madagascar.

Famille	Espèce	Endémicité	Particularité
Emballonuridae	*Coleura kibomalandy*	Oui	
	Paremballonura tiavato	Oui	
Hipposideridae	*Hipposideros commersoni*	Oui	
Miniopteridae	*Miniopterus aelleni*	Non	Sympatrie
	Miniopterus cf. *ambohitrensis*	Oui	Sympatrie
	Miniopterus gleni	Oui	Sympatrie
	Miniopterus griffithsi	Oui	Sympatrie
	Miniopterus griveaudi	Non	Sympatrie
	Miniopterus mahafaliensis	Oui	Sympatrie
	Miniopterus majori	Oui	Sympatrie
	Miniopterus sororculus	Oui	Sympatrie
Molossidae	*Chaerephon atsinanana*	Oui	Sympatrie et Synanthropique
	Chaerephon leucogaster	Non	Sympatrie et Synanthropique
	Mops leucostigma	Non	Sympatrie et Synanthropique
	Mops midas	Non	Sympatrie et Synanthropique
	Mormopterus jugularis	Oui	Sympatrie et Synanthropique
	Otomops madagascariensis	Oui	Sympatrie et Synanthropique
Pteropodidae	*Eidolon dupreanum*	Oui	
	Pteropus rufus	Oui	
	Rousettus madagascariensis	Oui	
Rhinonycteridae	*Paratriaenops furculus*	Oui	
	Triaenops menamena	Oui	
Vespertilionidae	*Hypsugo bemainty*	Oui	
	Myotis goudoti	Oui	Sympatrie
	Neoromicia malagasyensis	Oui	
	Neoromicia matroka	Oui	
	Neoromicia robertsi	Oui	
	Pipistrellus cf. *hesperidus*	Non	
	Pipistrellus hesperidus	Non	Sympatrie
	Pipistrellus raceyi	Oui	Sympatrie
	Scotophilus marovaza	Oui	Synanthropique

Tableau 4. Caractéristiques et particularités des chauves-souris collectées dans les autres îles de la zone SOOI (adapté d'O'Brien, 2011).

Localité	Famille	Espèce	Endémicité	Particularité
La Réunion	Molossidae	*Mormopterus francoismoutoui*	Oui	Synanthropique
Maurice	Molossidae	*Mormopterus acetabulosus*	Oui	
	Pteropodidae	*Pteropus niger*	Oui	Synanthropique
Mahé	Pteropodidae	*Pteropus seychellensis*	Oui	Synanthropique
Anjouan	Miniopteridae	*Miniopterus griveaudi*	Non	
	Molossidae	*Chaerephon pusillus*	Non	Synanthropique
Mayotte	Pteropodidae	*Pteropus seychellensis*	Oui	Synanthropique

3.3. Les petits mammifères terrestres

Chaque île du SOOI est par ailleurs connue pour abriter des rongeurs introduits, notamment, *Rattus rattus* et *Rattus norvegicus*. Cependant, Madagascar est l'une des seules îles du SOOI à héberger de nombreuses espèces endémiques. A ce jour 64 espèces de petits mammifères terrestres y ont été décrites dont 59 endémiques (Soarimalala & Goodman, 2013). La sous famille des Oryzorictinae représente la plus grande sous famille dont le genre *Microgale* est le plus diversifié et contient plus de 23 espèces. La plus grande partie des espèces de cette sous-famille est capable de vivre en sympatrie. Le **Tableau 5** décrit les principaux petits mammifères terrestres connus à Madagascar qui ont fait l'objet de notre étude.

Les petits mammifères terrestres indigènes jouent un rôle très important dans l'écosystème. Ils sont prédateurs d'insectes nuisibles ou ravageurs, disséminateurs de graines, ou servent de proies aux rapaces, carnivores et reptiles. A l'inverse, parmi les espèces introduites, les rats (*Rattus rattus*) sont source de nombreux problèmes. Ils sont responsables de la destruction des cultures agricoles et sont vecteurs de nombreuses maladies. A titre d'exemple *Rattus rattus, Mus musculus* et *Rattus norvegicus,* ainsi que 2 autres espèces introduites, *Suncus murinus* et *Suncus etruscus,* sont réputés vecteurs de la peste.

Tableau 5. Caractéristiques et particularités des petits mammifères terrestres collectées à Madagascar (adapté de Soarimalala & Goodman, 2013).

Famille	Sous-famille	Espèce	Endémicité	Particularité
Tenrecidae	Tenrecinae	*Hemicentetes semispinosus*	Oui	Sympatrie
		Setifer setosus	Oui	Sympatrie
		Tenrec ecaudatus	Oui	
	Oryzorictinae	*Microgale cowani*	Oui	Sympatrie
		Microgale dobsoni	Oui	
		Microgale fotsifotsy	Oui	
		Microgale gymnorhyncha	Oui	Sympatrie
		Microgale jobihely	Oui	
		Microgale longicaudata	Oui	Sympatrie
		Microgale majori	Oui	
		Microgale parvula	Oui	
		Microgale principula	Oui	
		Oryzorictes hova	Oui	
Soricidae	Soricinae	*Suncus murinus*	Non	Synanthropique
Muridae	Murinae	*Rattus norvegicus*	Non	Synanthropique
		Rattus rattus	Non	Synanthropique
		Mus musculus	Non	
Nesomyidae	Nesomyinae	*Eliurus majori*	Oui	
		Eliurus minor	Oui	Sympatrie
		Gymnuromys roberti	Oui	
		Nesomys rufus	Oui	Sympatrie

Malgré la diversité très élevée de chauves-souris et de petits mammifères terrestres dans le SOOI, la faune sauvage de cette région a été très peu explorée jusqu'à peu de temps en terme de réservoirs potentiels d'agents zoonotiques. Dans une étude préliminaire réalisée en 2012 par le CRVOI, sur les chauves-souris de Madagascar, des Comores et de La Réunion, une dizaine de nouveaux paramyxovirus a été détectée chez 5 espèces de chauves-souris insectivores. Ces paramyxovirus formaient un phylogroupe à part entière, proche du genre *Morbillivirus* sans qu'il soit possible de les rattacher complètement sur le plan phylogénétique (Wilkinson et al., 2012). Les taux d'infection élevés par ces nouveaux paramyxovirus Morbilli-related, leurs diversité et variabilité génétique (15 à 30% de variabilité génétique), ainsi que les spectres d'hôtes qui leur ont été associé marqué par un fort endémisme, en comparaison à d'autres études similaires dans le monde, nous ont alors conduit à émettre l'hypothèse des singularités écologiques régionales, agissant au niveau des échanges viraux entre hôtes. Dans ce contexte, l'objectif principal de mes travaux de thèse a été de déterminer quels sont les déterminants génétiques et écologiques qui peuvent rendre compte de tels taux d'infection par les paramyxovirus, d'une telle variabilité génétique et d'un tel spectre d'hôte dans la région du SOOI.

PARTIE II. TRAVAUX DE RECHERCHE

Dans cette partie, j'introduirais le contexte et les objectifs des programmes de recherche conduits au cours des 3 années (Janvier 2013-Décembre 2015).

Les résultats seront ensuite présentés en 3 chapitres (**chapitres 4 à 6**). Une partie de ces résultats est déjà publiée sous forme d'un article dans une revue internationale à comité de lecture, l'autre partie est structurée en 3 manuscrits soumises ou en cours de publication.

Une discussion générale des résultats obtenus, de leur signification ainsi que des perspectives envisageables par ces travaux clôturera ce manuscrit.

Présentation du sujet, des objectifs et questions de recherche

Contexte de l'étude

Comme abordé dans le **chapitre 3** de l'état de l'art, la région du SOOI est singulière par l'existence d'une multi-insularité fortement contrastée, à la fois au niveau géographique que géologique et de la faune sauvage qui y vit. Celle dernière est particulière dans la plupart des petites îles par une richesse spécifique relativement limitée par rapport à Madagascar ou à certaines zones continentales mais elle possède un niveau d'endémisme extrêmement marqué pour certains groupes d'animaux, notamment les chauves-souris et les petits mammifères terrestres (Goodman et al 2003 ; Yoder et al., 2005 ; Goodman et al., 2007; Goodman et al., 2008 ; Goodman, 2011). Nous avons fait l'hypothèse que cette biodiversité ne devrait pas être limitée aux seuls règnes animal et végétal; mais que le monde microbien, qui leur est inféodé, devrait l'être également. Ainsi, les spécificités écologiques des îles du SOOI associées à leur isolement géographique font de cette région un remarquable champ d'investigation sur (i) la dynamique évolutive des processus infectieux et des flux de pathogènes qui peuvent survenir au sein des communautés d'espèces, et (ii) des facteurs biotiques et abiotiques qui entrent en jeu dans les interactions hôte-pathogène, et des mécanismes macro-évolutifs mobilisés (Tortosa et al., 2012).

L'avènement des méthodes moléculaires a rendu possible des investigations menées aussi bien au niveau de l'écologie des hôtes que des agent infectieux, pour identifier des animaux réservoirs et inventorier les pathogènes potentiels qui leurs sont associés. Etudier la dynamique insulaire de transmission virale au sein d'une communauté d'hôtes pouvant être assimilées à des réservoirs et comprendre les forces évolutives en action au niveau macro-évolutif (Moss et al., 2012 ; Saluzzo et al., 2014) sont considères comme des étapes indispensables pour contextualiser "*in natura*" un évènement d'émergence s'il devait se produire un jour chez des populations humaines naïves.

Objectifs et questions de recherche

Deux modèles viraux ont été étudiés pour leur aptitude à transgresser les barrières d'espèces, un trait directement lié au risque d'émergence.

Les paramyxovirus (*Paramyxoviridae*) et les lyssavirus (*Rhabdoviridae*). Elles représentent deux familles virales très diversifiées et structurellement très proches (Assenberg et al., 2010 ; Drexler et al., 2012 ; Banyard et al., 2014). Plus spécifiquement les questions suivantes ont été posées :

i). Quelle est la dynamique de transmission virale inter-espèces chez les petits mammifères terrestres et les chauves-souris de la zone du SOOI ?

Cette étude a principalement porté sur les petits mammifères terrestres (ordre: Afrosorocidae) et volants (ordre: Chiroptera) endémiques de Madagascar, puis elle a été étendue aux rongeurs (ordre: Rodentia) de Mayotte, La Réunion et Madagascar. Des analyses phylogénétiques ont permis, d'une part, de mieux établir l'appartenance phylogénétique des paramyxovirus détectés aux *Unclassified Morbilli-Related viruses* (*UMRVs*), et d'autre part, de déterminer la façon dont pouvait opérer les flux de transmission au niveau intra- et inter-ordres, définissant ainsi les contours des différents spectres d'hôtes associés à ces *UMRVs* **(Chapitre 4**).

ii). Quel est le mécanisme macro-évolutif qui prédomine chez les *UMRVs* de chauves-souris et les facteurs environnementaux associés à la dynamique virale?

L'étude de consortia de chauves-souris réparties sur l'ensemble de l'île de Madagascar, représentant un ensemble de 35 espèces différentes et de leurs *UMRVs* inféodés, a permis de dégager, à l'aide d'analyses phylogénétiques (co-phylogénies) et statistiques (modèles linéaires généralisés), le mécanisme évolutif qui sous-tend la transmission interspécifique au sein des communautés de chauves-souris, et d'identifier certains facteurs biotiques et abiotiques qui influencent la relation entre les hôtes et les pathogènes (**Chapitre 5**).

iii). Quelle est la situation épidémiologique des lyssavirus de la région SOOI?

Pour investiguer la présence et caractériser la circulation des lyssavirus des chiroptères du SOOI, des chauves-souris de différentes espèces ont été collectées dans l'archipel des Comores, des Mascareignes, et des Seychelles, ainsi qu'à Madagascar. Les échantillons prélevés ont été analysés par la technique de référence RFFIT, et par biologie moléculaire pour la mise en évidence, respectivement, d'anticorps neutralisants anti-lyssavirus et de génomes viraux (**Chapitre 6**).

Chapitre 4. Étude de la dynamique d'infection des paramyxovirus chez les petits mammifères et les chauves-souris de la zone Sud-Ouest Océan Indien

4.1. Généralités

Les paramyxovirus appartiennent à une vaste famille qui ne cesse de grandir et de se diversifier. La famille *Paramyxoviridae* est divisée en deux sous familles: les *Paramyxovirinae* et les *Pneumovirinae* (**Annexe 1**).

La sous famille des *Paramyxovirinae* comprend 31 espèces classées en sept genres (ICTV, 2014): les *Respirovirus*, les *Rubulavirus*, les *Avulavirus*, les *Morbillivirus*, les *Henipavirus* et ceux nouvellement classés, les *Ferlavirus* et les *Aquaparamyxovirus*. Elle comprend entre autre, les virus de la rougeole (*measles*), des oreillons, (***mumps***) de la maladie de Newcastle (*Newcastle disease virus*), les parainfluenza, les virus *Hendra* et *Nipah*. Certains membres des *Paramyxovirinae* récemment émergents (par exemple *Menangle*, *Tioman*, *Beilong*, et *J-virus*) ne sont pas formellement phylogénétiquement reliés à ces 7 genres principaux et sont en phase de reclassification.

La seconde sous famille *Pneumovirinae* se décompose en deux genres, les *Pneumovirus* et les *Métapneumovirus,* qui comprennent cinq espèces. Elle comprend également des anciens et des nouveaux pathogènes pour l'homme et l'animal, tels que les virus respiratoires syncytiaux humains et bovins, ainsi que les métapneumovirus humain et aviaire.

Les paramyxovirus sont mondialement distribués avec un large spectre d'hôte important (Drexler et al., 2012). Les *Morbillivirus* ont un très large spectre d'hôte et sont retrouvés chez l'homme, les dauphins, les chauves-souris, les rongeurs, les dromadaires et les bovins (Drexler et al., 2012), alors que les genres *Avulavirus*, *Aquaparamyxovirus* et *Ferlavirus* n'ont jamais été retrouvés chez l'homme.

Measles, reste à ce jour le *Morbillivirus* le plus dévastateur dans le monde causant des millions de morts, particulièrement dans les pays en développement (Moss & Griffin, 2012).

Les virus *Hendra* et *Nipah* du genre *Henipavirus* ont causé des épidémies mortelles en Australie et en Malaisie en 1994 et 1999 respectivement, provoquant d'innombrables pertes humaines et animales (Chua et al., 2000 ; Field et al., 2001 ; Mahalingam et al., 2012).

Au niveau structural, les paramyxovirus sont des virus à ARN monocaténaire de polarité négative, dont le génome mesure entre de 15 à 18 kb (**Annexe 2**). La nucléocapside est constituée de l'ARN associé à la nucléoprotéine (NP), la phosphoprotéine polymérase (P), et la protéine (L). La protéine L est l'ARN polymérase, la protéine P facilite la synthèse d'ARN

et la protéine NP contribue à maintenir la structure génomique. La nucléocapside associée avec la protéine de matrice (M) garnissant l'intérieur de l'enveloppe du virion. L'enveloppe contient deux glycoprotéines, une protéine de fusion (F), qui favorise la fusion des membranes virales et de l'hôte cellulaire, et une protéine de fixation virale (hémagglutinine-neuraminidase (HN), l'hémagglutinine (H), ou la protéine (G). La protéine F doit être activée par clivage protéolytique, ce qui génère deux glycopeptides F1 et F2 maintenus ensemble par un pont disulfure, et qui expriment une activité de fusion membranaire (Lamb & Parks, 2007).

4.2. Approches expérimentales

Les résultats issus des investigations du CRVOI antérieures à mes travaux de thèse ont mis en évidence de nouveaux paramyxovirus et l'existence potentielle de mécanismes d'échanges viraux entre des hôtes endémiques particuliers à la région du SOOI (Wilkinson et al., 2012). Ces acquis ont été rendus possibles grâce à de nombreuses campagnes de collectes de micromammifères volants et terrestres vivant en isolement ou en communauté d'espèces. Ce sont au total plus de 140 chauves-souris (8 espèces), 333 petits mammifères terrestres endémiques de Madagascar (20 espèces) et 264 rongeurs introduits (4 espèces) qui ont été capturés entre 2010 et 2013, et criblés pour la mise en évidence de paramyxovirus. La détection de ces derniers a été effectuée par RT-PCR à l'aide d'un système pangénérique (Tong et al., 2008) capable d'amplifier la grande majorité des membres des genres *Respirovirus*, *Morbillivirus* et *Henipavirus*. Les analyses phylogénétiques réalisées (Mr Bayes, Beast, Mesquite) ont permis de retracer l'histoire évolutive de ces nouveaux virus et de quantifier les possibles évènements de saut d'espèces par taxon et par origine géographique.

Dans la première publication ci-après (Wilkinson et al., 2014), nous avons appliqué cette méthode pour caractériser en profondeur la dynamique d'infection et démontrer l'existence d'un flux viral intra-ordre particulièrement chez les Chiroptera, Afrosorocidae de Madagascar et Rodentia. Plus surprenant encore d'un point de vue écologique nous avons mis en évidence que ces flux de virus pouvaient être identifiés au niveau inter-ordre, notamment, entre Chiroptera ou Afrosorocidae et Rodentia collectés à Mayotte, La Réunion et Madagascar.

Annexe 5 : Par ailleurs, une étude phylogénétique et phylogéographique a été conduite afin de déterminer l'histoire évolutive des paramyxovirus des rongeurs de Tunisie. Un total de 784 petits mammifères composé de *Psammomys obesus*, *Meriones shawi* et *Ctenodactylus gundi* indigènes du Nord de l'Afrique et de l'espèce introduite *Rattus rattus* ont pu être analysés et comparés aux résultats issus en partie de mes travaux sur les animaux des régions du SOOI. Les résultats de ces travaux constituent la seconde publication ci-dessous et soumise (Ghawar et al., 2015 en soumission).

4.3 Etude de la dynamique d'infection au sein des micromammifères de la zone SOOI. Résultats présentés dans la publication ci-après

Journal of Virology

Highly Diverse Morbillivirus-Related Paramyxoviruses in Wild Fauna of the Southwestern Indian Ocean Islands: Evidence of Exchange between Introduced and Endemic Small Mammals

David A. Wilkinson, Julien Mélade, Muriel Dietrich, Beza Ramasindrazana, Voahangy Soarimalala, Erwan Lagadec, Gildas le Minter, Pablo Tortosa, Jean-Michel Heraud, Xavier de Lamballerie, Steven M. Goodman, Koussay Dellagi and Herve Pascalis

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Highly Diverse Morbillivirus-Related Paramyxoviruses in Wild Fauna of the Southwestern Indian Ocean Islands: Evidence of Exchange between Introduced and Endemic Small Mammals

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ABSTRACT

The *Paramyxoviridae* form an increasingly diverse viral family, infecting a wide variety of different hosts. In recent years, they have been linked to disease emergence in many different animal populations and in humans. Bats and rodents have been identified as major animal populations capable of harboring paramyxoviruses, and host shifting between these animals is likely to be an important driving factor in the underlying evolutionary processes that eventually lead to disease emergence. Here, we have studied paramyxovirus circulation within populations of endemic and introduced wild small mammals of the southwestern Indian Ocean region and belonging to four taxonomic orders: Rodentia, Afrosoricida, Soricomorpha, and Chiroptera. We report elevated infection levels as well as widespread paramyxovirus dispersal and frequent host exchange of a newly emerging genus of the *Paramyxoviridae*, currently referred to as the unclassified morbillivirus-related viruses (UMRVs). In contrast to other genera of the *Paramyxoviridae*, where bats have been shown to be a key host species, we show that rodents (and, in particular, *Rattus rattus*) are significant spreaders of UMRVs. We predict that the ecological particularities of the southwestern Indian Ocean, where small mammal species often live in densely packed, multispecies communities, in combination with the increasing invasion of *R. rattus* and perturbations of endemic animal communities by active anthropological development, will have a major influence on the dynamics of UMRV infection.

IMPORTANCE

Identification of the infectious agents that circulate within wild animal reservoirs is essential for several reasons: (i) infectious disease outbreaks often originate from wild fauna; (ii) anthropological expansion increases the risk of contact between human and animal populations and, as a result, the risk of disease emergence; (iii) evaluation of pathogen reservoirs helps in elaborating preventive measures to limit the risk of disease emergence. Many paramyxoviruses for which bats and rodents serve as major reservoirs have demonstrated their potential to cause disease in humans and animals. In the context of the biodiversity hot spot of southwestern Indian Ocean islands and their rich endemic fauna, we show that highly diverse UMRVs exchange between various endemic animal species, and their dissemination likely is facilitated by the introduced *Rattus rattus*. Hence, many members of the *Paramyxoviridae* appear well adapted for the study of the viral phylodynamics that may be associated with disease emergence.

Wild rodents and bats together comprise more than 60% of all known mammalian species and have been found to carry many zoonotic viruses (1). Recently, these groups of mammals have been shown to host a broad spectrum of novel paramyxoviruses (2–8). These data have broadened our knowledge of the host spectrum of *Paramyxoviridae*, a diverse viral family which is currently divided into two main subfamilies: the *Pneumovirinae* and the *Paramyxovirinae*. Members of the *Paramyxoviridae* include the causative agents of the human diseases mumps, measles and other respiratory tract infections due to *Parainfluenza* viruses and metapneumoviruses, as well as a large number of viruses associated with disease in animals, such as Newcastle disease virus, canine distemper virus, rinderpest virus, and others. Additionally, paramyxoviruses have been linked to a number of recent emerging or reemerging disease epidemics with high mortality rates (9). Of these, henipaviruses (*Paramyxovirinae*) have emerged as human pathogens from fruit bat populations since 1994 in Bangladesh, Malaysia, and Australia (10, 11). Additionally, a novel rubulavirus-related virus, similar to those known to be hosted by fruit bats, has been described with the capacity to cause illness in humans (12), showing how as-yet unknown *Paramyxoviridae* in wild animal reservoirs may pose an important health risk.

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A virus's reservoir consists of often-complex host communities that participate in its propagation and maintenance (13). Dynamics of individual host populations contribute to viral prevalence (14, 15), circulation, and dispersal (16); however, virus adaptation to its host can limit the potential for transmission between species (17, 18). The dynamics of viruses associated with their phylogenetics have been shown to be driven by a large number of complex and interacting factors (19–21), including the genetic diversity, ecology, and population dynamics of their host communities.

The southwestern Indian Ocean (SWIO) region is recognized as part of a biodiversity hot spot (22). In Madagascar, endemic mammalian taxa (including all native terrestrial and a significant percentage of volant species) have colonized the island independently since its separation from the Gondwana supercontinent more than 150 million years ago (23). In contrast, the smaller and much younger oceanic islands (mainly of volcanic origin) located around Madagascar (Mascarene, Comoros, and Seychelles archipelagos) host a limited number of mammalian species, and all of the terrestrial forms have been introduced (24, 25). The islands of the SWIO have been subjected to rapid anthropological expansion resulting in the dispersal and invasion of nonendemic rats and mice of the family *Muridae* and shrews of the family *Soricidae* to these isolated island ecosystems (26, 27). On virtually all of the SWIO islands, these introduced small-mammal populations occur in often dense populations, and on Madagascar, they compete with and displace native animal communities (28), potentially promoting the exchange of introduced infectious agents. These aspects make the SWIO an ideal location for the study of pathogen exchange (29).

In a previous study (3), we reported paramyxovirus infection in SWIO bats. Here, we have expanded our investigation into the diversity of this viral family within bats and other small mammals of Madagascar and other SWIO islands. We find that novel and unclassified morbillivirus-related paramyxoviruses (UMRVs) are commonplace within regional small mammals. Important differences between the morbillivirus-related and henipavirus-related virus models are observed, and detailed phylogenetic analyses suggest a major role for introduced *R. rattus* in diffusing UMRVs between different mammalian orders and different islands. These results highlight that UMRVs are a good model for understanding viral dynamics within wild fauna, especially between rodents and bats, two major reservoirs of zoonotic pathogens.

MATERIALS AND METHODS

Field work and sample collection. A total of 597 small, nonflying mammals were captured on Madagascar at two sites over the course of 3 years (2010 to 2012), once on Mahé (Seychelles) (June 2011) and once on Mayotte (Comoros) (July 2012), and from different sites on La Réunion (September 2012 to March 2013). A total of 140 bats were collected from several locations in Madagascar in 2012. Details on animal capture techniques are provided in the Text S1 in the supplemental material, and those on sampling locations are provided in Table S3.

Ethics statement. All animals were manipulated in accordance with guidelines for the handling of wild mammals (30). This study benefitted from sampling efforts conducted in the context of an ongoing international long-term project to catalogue the terrestrial vertebrate fauna of Madagascar based on voucher specimens (31). All protocols followed the terms of research permits (see Acknowledgments) issued by national authorities: Ministère des Forêts et de l'Environnement, Madagascar National Parks, Département de Biologie Animale (Madagascar); Direction de l'Environnement, de l'Aménagement et du Logement (France); and the Seychelles Bureau of Standards (Seychelles).

Laboratory work. (i) Nucleic acid preparation. Samples of approximately 1 to 2 mm^3 of kidney, spleen, and lung tissues were dissected on sterile ice from each individual animal. These tissue samples were then combined in 750 μl of Dulbecco's modified medium (Gibco, USA) containing 2- by 3-mm tungsten beads and homogenized for 2 min at 25 Hz in a TissueLyser (Qiagen). Homogenized organ samples were then clarified by centrifugation at 10,000 × *g* for 5 min. Two hundred μl of the clarified medium was biologically inactivated in 200 μl of AVL buffer (Qiagen), and total nucleic acids were extracted using the EZ1 virus V2 minikit in an EZ1 biorobot (Qiagen). Total nucleic acids were reverse transcribed in the presence of random hexameric primers using the GoScript reverse transcription kit (Promega) according to the manufacturer's instructions.

(ii) PCR screening. cDNA from each sample was screened using a seminested PCR system that targets a partial sequence (~490 bp) of the L polymerase gene of respiroviruses, morbilliviruses, and henipaviruses (RMH) as described by Tong et al. (32) and previously exploited in similar studies (2–6). Negative controls were routinely incorporated, and PCRs were repeated independently to ensure no cross-contamination when using nested PCR protocols.

(iii) Statistical analyses. Differences in detected prevalence (defined as the proportion of animals tested from which the sequence of the RMH PCR product obtained had significant homology to known *Paramyxovirus* sequences in BLAST) were statistically analyzed using a two-tailed Fisher's exact test. $P < 0.05$ was considered statistically significant.

(iv) Sequencing. PCR cDNA products of the approximate anticipated size (450 to 500 bp) were purified using the Qiagen PCR purification kit and cloned into the pGEM-T vector system (Promega). Cloned PCR products were Sanger sequenced (Genoscreen) using M13 standard sequencing primers. The sequence quality of individual reads was assessed, and all sequences were processed using the Geneious Pro software package (33). DNA sequences obtained from at least three independent bacterial clones were aligned to correct for the majority of sequencing or PCR-introduced errors. Coinfection was defined when sequences from the same animal showed greater than 5% genetic dissimilarity; in these cases, at least three clones of each different sequence were obtained. Primer sequences were trimmed from the finalized sequences.

(v) OTU-based family-level phylogeny. In order to classify the detected paramyxoviruses, viral family-level phylogenetic analyses were performed. The search parameters "Taxonomic classification: *Paramyxoviridae*" and "Any Field: polymerase" were used in GenBank (15 December 2013) to generate the final comparative sequence data set. In total, 1,816 sequences were assessed for their compatibility via pairwise alignment against a reference sequence from the *Henipavirus* genus (JN255806). The Geneious Translational alignment tool, using the default ClustalW cost matrix, was used to align all compatible sequences. Sequences were trimmed to remove any free end gaps or entirely removed from the analysis if the obtained alignment did not provide at least 420 bp of non-gap overlap, leaving 1,192 sequences for phylogenetic analysis (final length after trim, excluding gaps, 438 bp). Internal gaps were permitted. Operational taxonomic units (OTU) were defined using mothur (34) with a genetic distance cutoff of 10%, generating 196 consensus sequences that spanned all known, classified, and unclassified paramyxovirus genera. Representative sequences were selected using mothur for each defined OTU, which were then used in the final phylogeny (see Fig. 3).

(vi) Sequence polymorphism. DNAsp v.5 (available at http://www.ub.edu/dnasp/) was used to calculate the estimates of genetic diversity, Pi and Theta, from subsets of the final nucleotide alignments, separated by host species.

(vii) UMRV (and henipavirus) phylogeny. Individual sequences from OTUs falling within the UMRV clade were extracted from the original sequence alignment and used for further phylogenetic analysis (see

Fig. 4 and 5; also see Fig. S2 in the supplemental material). *Salem virus* (JQ697837) was included as an outgroup reference sequence.

(viii) Phylogenetic analysis. All presented phylogenies were constructed using the same methodologies; from aligned sequence data sets, jModelTest v2.1.2 (35) identified GTR+I+G as the most appropriate substitution model for all phylogenetic analyses in BEAST (36). Parameter estimates for different clock models were assessed in Tracer v1.5.0 (36), and a strict molecular clock was used for all further analyses. An additional three replicates of 100,000,000 iterations then were performed using these optimal parameters. Trees and parameter estimate files for all independent replicates then were combined using LogCombiner (BEAST package), assigning a 10% burn-in, which left only converged parameter estimates from each repeat. The obtained effective sample size values for each parameter were all superior to 200. The final phylogeny was generated using TreeAnnotator, and trees were manipulated using FigTree v1.4.

(ix) Cartographic data. Distributions of hosts were downloaded from the IUCN Global Mammal Assessment (http://www.iucnredlist.org/). Maps were processed in the Quantum GIS 2.0.1 (Dufour) software package.

(x) Ancestral-state reconstruction. Bayesian phylogenies from each of the preceding clade-level analyses were resampled, and the resulting 4,000 trees were imported into Mesquite (version 2.75; http://mesquiteproject.org/mesquite/mesquite.html). Geographical origins and mammalian host categories were attributed to each sequence as discrete state characters. The number of reconstructed trait changes was calculated using the unordered parsimony assumption and averaged for each ordinal category and over all trees, as described in reference 4.

Nucleotide sequence accession numbers. All sequences used for the present analyses have been deposited in GenBank under the reference numbers KF245939 to KF246061, KF408256 to KF408261, and KF928225 to KF928265.

RESULTS

Sampling across the SWIO islands yielded a wealth of animal samples. A description of the animal species subject to trapping activities is provided in Text S2 in the supplemental material. A molecular epidemiology survey for paramyxoviruses was carried out on 732 samples from four different animal orders, Chiroptera, Afrosoricida, Rodentia, and Soricomorpha, collected over a period of 3 years (2010 to 2012) in the SWIO region, specifically Madagascar, La Réunion, Mayotte (Comoros), and Mahé (Seychelles). All screening data are summarized in Fig. 1.

Madagascar. A total of 391 small nonflying mammals belonging to three orders (Rodentia, Afrosoricida, and Soricomorpha) and 22 different species were sampled at two sites on Madagascar. These included endemic Nesomyidae rodents (*Eliurus*, *Gymnuromys*, and *Nesomys*) and Tenrecidae tenrecs (*Microgale*, *Oryzorictes*, *Hemicentetes*, *Setifer*, and *Tenrec*), as well as introduced Muridae rodents (*Rattus*) and Soricidae shrews (*Suncus*). The species captured at each of the two sites varied, although the species diversity was comparable. The numbers of taxa captured at each site showed little temporal variation during the different field visits, suggesting homogenous sampling between years; however, a larger number of *R. rattus* specimens were collected in Lakato in 2012 than in the previous years.

Of the Malagasy nonflying mammals screened for paramyxoviruses, 25% (98/391) tested positive. Shrew tenrecs (Tenrecidae) belonging to the genus *Microgale*, for which the majority of sampled animals were represented by *M. cowani* ($n = 73$) and *M. dobsoni* ($n = 54$), showed the highest rate of paramyxovirus detection (40% positive; 69/172). Of these, four animals were coinfected with at least two different paramyxovirus strains (see Table S1 in the supplemental material). Paramyxoviruses were also detected in rodents, including *R. rattus* (30% positive; 16/54) and *Eliurus minor* (11% positive; 12/111). Variation in paramyxovirus infection levels was observed over the 3 years of collections on Madagascar, most markedly in populations of *M. cowani*, where differences between consecutive years were strongly significant ($P < 0.001$ for 2010 to 2011 and $P = 0.001$ for 2011 to 2012) and paramyxovirus infections peaked in 2011, with 85% of animals testing positive (Fig. 2).

Of the 140 Malagasy bats screened, a total of 41 belonging to eight bat species endemic to SWOI (*Chaerephon leucogaster*, *Miniopterus griveaudi*, *Mops leucostigma*, *Mormopterus jugularis*, *Myotis goudoti*, *Otomops madagascariensis*, *Triaenops menamena*, and *Vespertilionidae* spp.) and from four bat families (Hipposideridae, Miniopteridae, Molossidae, and Vespertilionidae) tested positive for paramyxoviruses.

Animals not endemic to other SWIO islands. Nonendemic small mammals collected outside Madagascar included *R. rattus*, *R. norvegicus*, and *Suncus murinus* on La Réunion and *R. rattus* on Mayotte and Mahé; paramyxoviruses were detected at each of these locations (Fig. 1), with *Rattus* spp. showing the highest proportion of positive animals (22%; 42/187).

Genetic and phylogenetic analyses. Phylogenetic analysis based on OTUs was sufficient to generate a well-supported, family-level phylogeny containing monophyletic groups for all main *Pneumovirinae* and *Paramyxovirinae* genera (Fig. 3). From this phylogeny, 10 clades were defined based on well-supported nodes (posterior probability [PP] > 0.8) that provided definitions at the taxonomic level of genus based on current taxonomic classifications. All of the 177 paramyxovirus sequences from the SWIO were members of a single genus-level group (UMRVs).

Absolute levels of genetic divergence were comparable between henipaviruses, morbilliviruses, and the UMRVs (see Fig. S1 in the supplemental material). Partial L-gene sequence divergence was calculated for those host species groups that were well represented in global sequence data and of interest for this study (see Table S2 in the supplemental material). Interestingly, less genetic divergence was observed between viral sequences originating from host species of different bird and mammalian orders (Aves, Afrosoricida, Rodentia, Soricomorpha, and Scandentia) than between viral sequences originating from within the Chiroptera, suggesting that bats can be infected by a broader range of paramyxoviruses than other hosts (Table 1). However, within the UMRV group, the genetic diversity was comparable between viral sequences from rodents and bats (Table 2).

Further phylogenetic analyses of all sequences that fell within the UMRV OTU-defined subgroups indicate that the detected paramyxoviruses comprise a certain level of host specificity at the level of host order (Fig. 4; also see Fig. S2 in the supplemental material). Two well-supported clades contain the majority of sequences originating from rodents, an additional two clades include most sequences originating from bats, and one final clade contains the greater part of sequences from tenrecs. Geographical clustering within the phylogeny was less apparent apart from viruses found in Afrosoricida, which are endemic to Madagascar (Fig. 5; also see Fig. S2). Cartographic analysis of the distributions of those species identified as hosts of the different UMRV groups (Fig. 4) suggested the global dispersal of many of these viral agents.

Members of the UMRVs included the Jeilam viruses (J, Beilong, and Tailam), viruses of rodent origin isolated in Australia

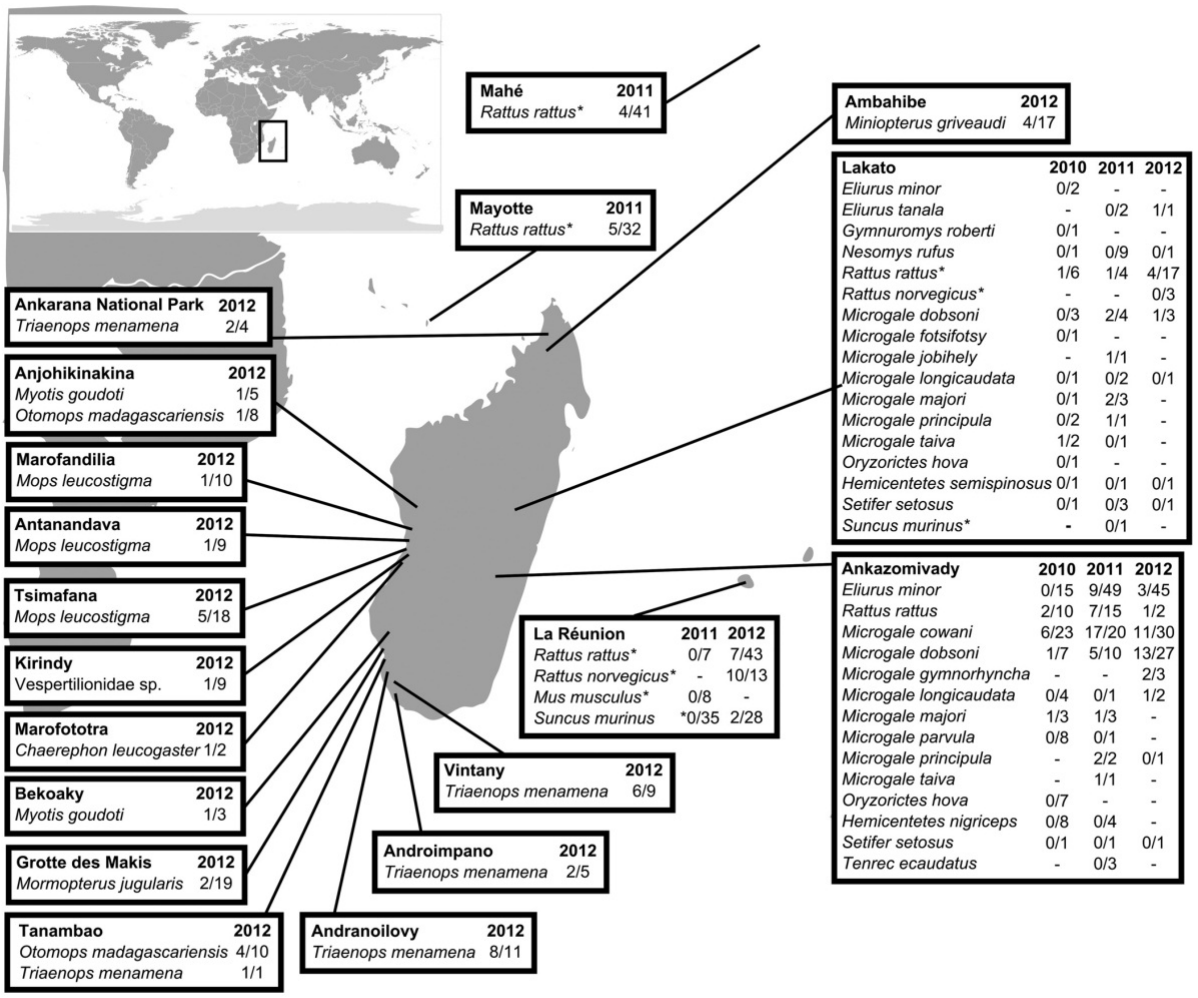

FIG 1 Paramyxovirus detection at different sampling sites in Madagascar and the SWIO. Numbers of positive animals, total numbers of sampled animals, and the year of capture are indicated for each sampling site. Species names preceded by an asterisk represent introduced taxa.

and China. Genetic and virological evidence has previously demonstrated that these viruses belong to the same taxonomic group (37–39). Interestingly, a strongly supported phylogenetic substructure separated Beilong and J viruses (see Fig. S2 in the supplemental material). The well-supported monophyletic group uniting these two viruses (PP = 1.0), designated rodent group 2 in Fig. S2 in the supplemental material, contained a further 71 paramyxovirus sequences, which were obtained from rodents and other small mammals from across the globe. Further well-supported substructures within rodent group 2 demonstrated differences in geographical distribution and host species association; for example, one subgroup contained only sequences obtained from the southern African rodent *Rhabdomys pumilio* (Muridae), whereas Beilong and Tailam viruses branched closely with *Rattus* species-derived sequences from the SWIO.

Ancestral-state reconstructions of UMRVs. Ancestral-state reconstructions were used to estimate the number of viral host switches and geographical exchanges based on the phylogeny presented in Fig. 2. These data are presented in donor/acceptor probability heatmap form in Fig. 4. Host-switching events were predicted to be most frequent between closely related species and between species belonging to the same order. *R. rattus* was predicted to participate in the largest number of host-switching events, being both the principle donor and principal acceptor species. *R. rattus* was also predicted to be involved in the majority of host exchanges that occurred between distantly related animal orders. Notably, elevated numbers of bidirectional viral exchanges between *R. rattus* and the Tenrecidae (*Microgale* spp.) were predicted to have occurred, and bats were also predicted to have received paramyxoviruses from *R. rattus*.

The geographical flux of paramyxoviruses was predicted to have occurred at least five times more frequently between Africa and the SWIO than any other continental regions. Exchange was also predicted to be frequent between SWIO islands, in particular between Madagascar, La Réunion, and the Comoros. It is important to note that our ancestral-state reconstructions could be in-

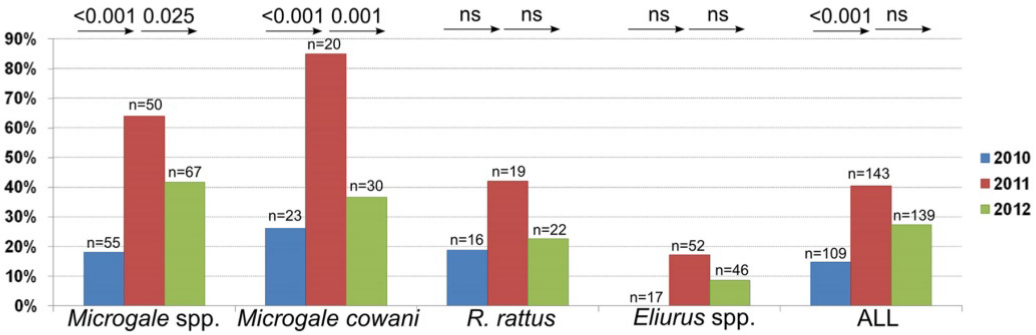

FIG 2 Temporal variation of detected paramyxovirus infection levels in animals from Madagascar. The number of paramyxovirus-positive animals is indicated in percentages. The total number of animals tested for each year is indicated above the bars. Colors are used to highlight the year of sampling, as described in the legend. *P* values displayed above each graph indicate the significance of differences between consecutive years (two-tailed Fisher's exact test; ns, not significant).

fluenced by a possible overrepresentation of paramyxovirus sequences from the SWIO; however, it should be noted that other large-scale screening efforts across the world have also contributed considerable data (2–6), originating from both rodents and bats and distributed across most known paramyxovirus genera (see Table S3 in the supplemental material). The limitations of similar analyses have been discussed in detail elsewhere for paramyxoviruses (4) and lyssaviruses (40).

For comparative purposes, similar analyses were performed on sequences from the henipavirus and unclassified henipavirus-related virus (UHRV) genus of the *Paramyxoviridae* (see Fig. S3 in the supplemental material). Here, host exchanges corresponding to human disease emergence via pigs and horses could be reliably predicted from the generated phylogeny, and geographical exchanges predicted an African origin for the henipaviruses, as well as large-scale circulation on the African continent, in agreement with other published literature on henipavirus biogeography (4, 8).

DISCUSSION

We have shown that many lineages of *Morbillivirus*-related paramyxovirus are in active circulation between islands in the SWIO. Bats and terrestrial small mammals play host to a number of highly divergent paramyxovirus strains belonging to this UMRV group.

Large numbers of paramyxovirus-positive animals were detected in this study. Endemic *Microgale* spp. and *Triaenops menamena* were identified as important paramyxovirus hosts, where 40% and 63% of animals, respectively, tested positive. In regional populations of introduced *Rattus* spp., this figure was

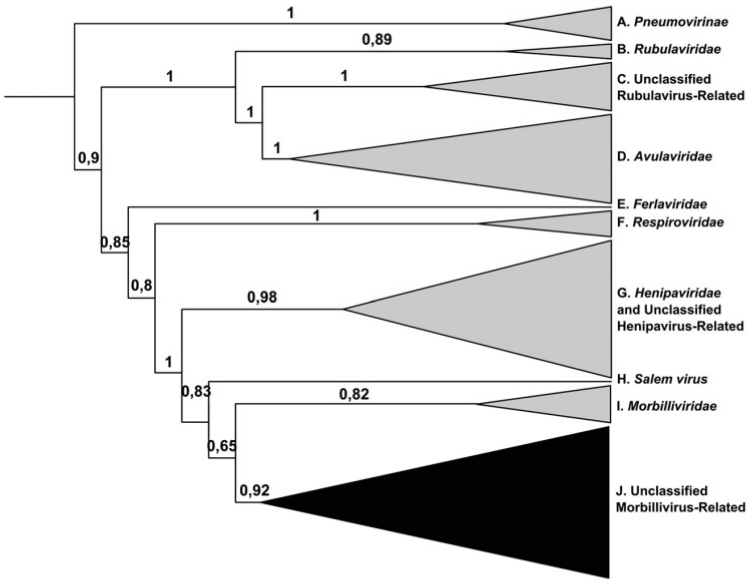

FIG 3 Paramyxovirus clades of the SWIO. (A) OTU-based family-level phylogeny of paramyxoviruses. The presented phylogeny is a proportional, ordered representation of data generated in BEAST. Relevant posterior probabilities (>0.5) are shown above each branch for clarity. Sequences are grouped into functional clades based on node support and height (see the text). Each functional group is assigned a letter (A to J). All sequences obtained within this study fell within group J, which is highlighted in black.

TABLE 1 Genetic diversity of the *Paramyxoviridae* by host order[a]

Parameter	Viral diversity in host order(s):			
	Aves	Chiroptera	Rodentia	Scandentia, Soricomorpha, and Afrosoricida
Pi (SD)	0.21123 (0.01385)	0.38181 (0.00730)	0.25410 (0.00947)	0.32354 (0.01149)
Theta	0.32854	0.35262	0.26267	0.33052

[a] Sequences from mammalian orders Scandentia and Soricomorpha are grouped with sequences from Afrosoricida due to the small number of viral sequences originating from animals of these orders. Pi is an estimation of nucleotide diversity, and Theta is equivalent to the Watterson estimator of the population mutation rate.

20%. A recent study (4) reported that *R. rattus* in Gabon and Thailand and *R. norvegicus* in Germany had low levels of infection (0/113, 1/49, and 0/131, respectively) when employing similar detection methods. However, the same study reported elevated numbers of paramyxoviruses in southern African *Rhabdomys pumilio* populations (17% of 512 animals tested). Additionally, another study conducted in Zambia has recently reported comparably high paramyxovirus infection levels in rodent (19%) and shrew (40%) populations (7). It is also of note that no henipavirus-related viruses were detected as part of this study despite the existence of serological evidence for the circulation of henipaviruses in Madagascar (41); however, members of this viral genus most often are associated with Pteropodidae fruit bats, which were absent from our samples.

The genetic diversity of partial L-gene sequences studied here is notably high, which is suggestive of the circulation of a large number of previously uncategorized viral strains (4). Additionally, multiple viral lineages could be identified within individual hosts, suggesting a level of compatibility between infecting viral strains. The overall observed genetic diversity of all paramyxoviruses per host was highest within the Chiroptera, suggesting that bats host a broader range of paramyxoviruses than terrestrial small mammals, in agreement with a recent hypothesis that bats are more permissive to viral infection than rodents (1). However, within the UMRV genus the level of viral diversity was similar between rodents and bats, showing that the relative importance of each host species likely is viral genus specific.

Different factors that may drive the observed levels of high genetic diversity of paramyxoviruses include the following: (i) the inherent genetic drift associated with many RNA viruses (42), which alone may be insufficient to explain high genetic variation between closely related viral lineages; (ii) coinfection within individual hosts, which is a "prerequisite for genetic exchange between different pathogen species or strains" (43), was observed in this and other studies of novel *Paramyxovirus* genera (2–4), and the possibility of recombination events between highly similar viruses should not be excluded, although their observation is rare for members of the *Paramyxoviridae*; and (iii) viral adaptation due to exchange within multihost systems (18).

The observed phylogenetic structuring of a divergent viral population of Jeilam-related viruses suggests that these closely related lineages have followed separate ancestral paths, generating a population structure similar to that of previously reported viral metapopulations (44, 45). Furthermore, analysis of the overall sequence diversity within well-defined *Paramyxovirus* genera suggests that the studied small fragment (~450 bp) is prone to genetic diversification compared to other loci of the paramyxovirus genome (46). Thus, in the absence of further virological or genetic data, the taxonomy of these viruses remains imprecise. In contrast, the observed genetic diversity, in association with considerable data originating from sources across the globe, has proven efficient for tracing the evolutionary paths of different viral lineages.

Using previously proposed terminology (13), the abundance of observed paramyxovirus infections suggests that animals of the SWIO islands constitute a putative paramyxovirus maintenance community. We predict, based on the reported virus-host interactions, that interspecies contacts are frequent within the natural ecosystems of Madagascar, in turn promoting what has elsewhere been referred to as a disease hot spot (22). For example, within 125 km of our two study sites, 12 *Microgale* species occur in syntopic and densely packed ecosystems (47). Thus, a considerable number of phylogenetically closely related host species, as well as animals of different mammalian orders, interact within dense and closed biotic systems, and host-specific adaptation appears to impose few barriers to viral host switching, resulting in frequent viral spillover and persistence within a multispecies community.

Viral persistence within a classical model source/sink community can be accompanied by genetic adaptation that establishes efficient virus-host interactions (17, 48); this may eventually create an evolutionary barrier that inhibits further host switching and results in the establishment of a new source population. This evolutionary process becomes complex for multihost ecosystems that exhibit frequent bidirectional host switching, such as that observed on Madagascar. Numerous factors will influence the efficiency of viral spreading within any studied community, including (i) viral and host abundance, (ii) host fitness, (iii) infection cost to the host, and (iv) the frequency and mode of transmission events (48). Infection dynamics within reservoir populations also directly influence viral epidemiology, with peaks of infection resulting in increased viral transmission (16). Here, the proportion of paramyxovirus-positive *Microgale cowani* increased 4-fold be-

TABLE 2 Genetic diversity within the UMRV genus per host group[a]

Parameter	Genetic diversity in:			
	Chiroptera	*Rattus* spp.	Rodentia	Afrosoricida (Tenrecidae)
Pi (SD)	0.28304 (0.00637)	0.24013 (0.01689)	0.28515 (0.00767)	0.22637 (0.00964)
Theta	0.27860	0.23846	0.25717	0.22740

[a] Pi is an estimation of nucleotide diversity, and Theta is equivalent to the Watterson estimator of the population mutation rate.

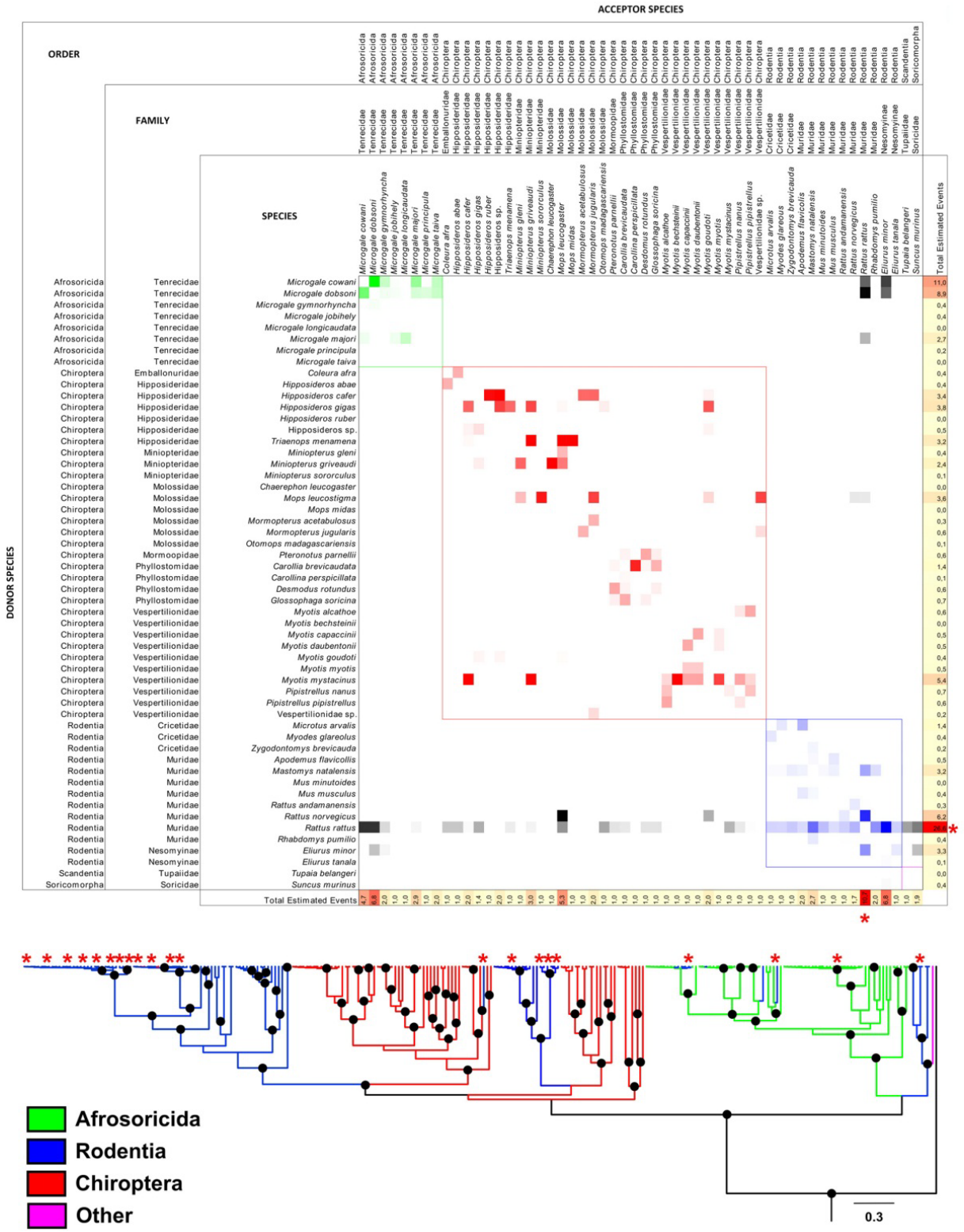

FIG 4 Ancestral-state reconstructions: host switching. The number of estimated host-switching events between species, based on parsimony analyses in Mesquite, are represented in tabular heatmap form. The presented phylogenetic tree is identical to that in Fig. 5 and Fig. S2 in the supplemental material and presents the most likely arrangement of the phylogenies used to estimate host-switching numbers. Dots represent nodes with posterior support greater than 0.9. Colors denote host-species order throughout. Red stars indicate positions of *R. rattus* in the phylogeny and the table.

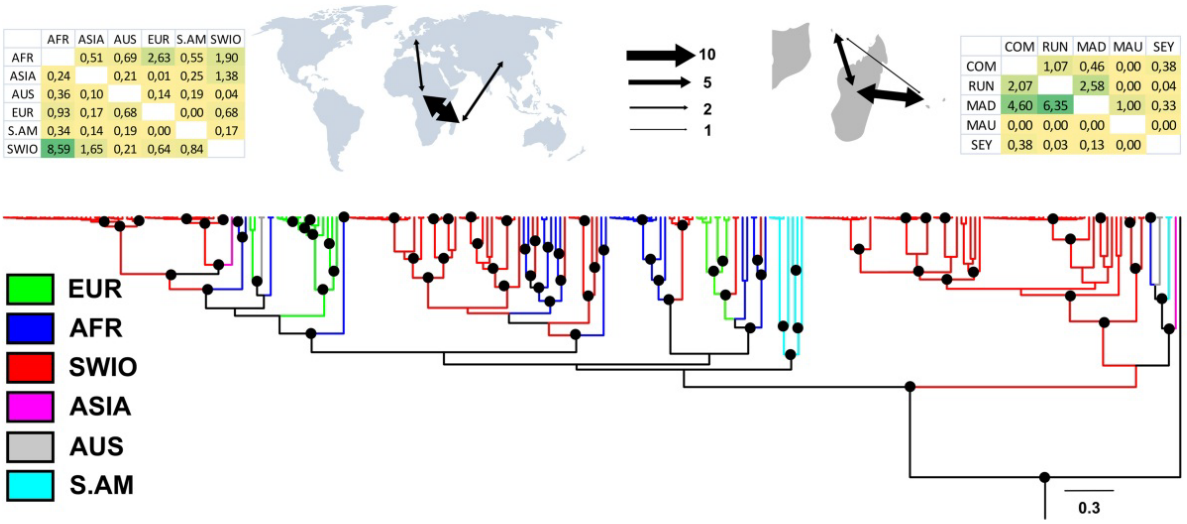

FIG 5 Ancestral-state reconstructions: geographical exchange. The number of estimated geographical switching events between sites based on parsimony analyses in Mesquite are represented in tabular form and are heatmap colored based on the estimated frequencies. The presented phylogenetic tree is identical to that in Fig. S2 in the supplemental material and Fig. 4 and presents the most likely arrangement of the phylogenies used to estimate geographical exchange numbers. Dots represent nodes with posterior support greater than 0.9. Colors depict continental origins.

tween 2010 and 2011, coinciding with the detection of closely related viral lineages in other host species. Spillover is a critical process for understanding zoonotic transmission but has rarely been documented in wild animal populations.

In addition to circulation within insular ecosystems, viral reservoirs can be established over large host communities, aided by their widespread geographical dispersal. From our data, the estimated levels of UMRV exchange between SWIO islands were high compared to global transmission levels, implying active host-associated dispersal between the African continent, Madagascar, and other SWIO islands.

Bats are theorized to play crucial roles in the processes of viral evolution that may lead to disease emergence (49) and are important hosts for numerous paramyxoviruses. Unlike henipaviruses, whose geographical distribution can be largely explained by the dispersal of Pteropodidae fruit bats (50), the observed geographical distribution of UMRVs is unlikely to be attributed uniquely to the dispersal of those bat species from which these viruses have been detected and most likely relies on interaction with other animal hosts. The data presented here indicate an important role for *R. rattus* in the transmission of UMRV paramyxoviruses and highlight these animals as potential intermediates in the dissemination of infectious agents that are endemic or otherwise isolated that facilitate disease emergence. The global dispersal of *Rattus* and their associated biota have been studied in many different contexts (25, 51) and have particularly important implications within the SWIO (52), where many mammal species are endemic, particularly on Madagascar, and at risk from both macro- and microorganism invasions, and where active commercial trade results in considerable and largely uncontrolled exchange (24, 53).

Within the insular ecosystems of the SWIO, paramyxovirus infection provides an excellent model for the study of both viral population dynamics and biogeography. Geographical exchange and host-switching events are common, likely occurring over short time scales due to some ecological factors that promote gene flow within an expansive viral maintenance community. In this context, *Rattus* play the dual role of a viral maintenance population and spreaders. Further virological investigation into the evolutionary implications of our observations may go a long way to developing an understanding of viral evolution within complex multihost communities.

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We have no conflicts of interest in relation to the submitted work to declare.

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Supplementary Material:

Supplementary Figure and Table Legends:

Supplementary Table S1: Paramyxovirus co-infections. Highlighted are animals from which multiple paramyxovirus L-gene sequences could be obtained, that showed clearly divergent sequences. Multiple discrete sequences are suggestive of viral co-infections.

Supplementary Table S2: Genbank accession numbers are presented for paramyxovirus sequences originating from bats, rodents or other small mammal species that were of interest to this study. Sequences are grouped into the major clades of the paramyxovirus family, based on family level phylogenies presented in main text Figure 2. Host-species names and the number of sequences originating from that species are shown for each group. Sequences generated as part of this study are underlined.

Supplementary Table S3: Description of capture sites, dates and species.

Supplementary Figure S1: Graphical representation of the genetic similarity (% identity) between different functional clade groups of the paramyxoviridae (see Figure 2). Numbers below the diagonal are the mean identities between all individual members of the compared groups. Numbers above the diagonal are the corresponding standard deviations of sequence identities. The indicated color scale of green to red highlights strong to weak sequence conservation.

Supplementary Figure S2 (previous page). Phylogeny of all sequences belonging to the UMRV group. Groups are defined by majority host-species. Red stars indicate the positions of *Beilong* (DQ100461) and *J-viruses* (NC007454, AY900001) in the phylogenetic tree.

Supplementary Figure S3. Geographical distributions of paramyxovirus-positive species. The potential extent of each group presented in Supplementary Figure S2 is

calculated from the geographical distribution of its hosts, according to the IUCN database (http://www.iucnredlist.org/), and is indicated in dark red to the right. Light red is used for locations outside of Madagascar for the “Malagasy Afrotheria Group”, all sequences within this phylogenetic group originated from Madagascar including the minority of sequences that were detected in R. rattus. There is no evidence that viruses sharing sequence homology to the Malagasy Afrotheria Group exist outside of Madagascar.

Supplementary Figure S4. Ancestral state reconstructions – *Henipaviruses* and *Unclassified Henipa-related Viruses*. Ancestral host-species (A) and geographical (B) states were reconstructed and used to predict switching events in Mesquite based on 4000 raw trees of the *Henipavirus* phylogeny generated in BEAST. Host switching events that are linked with disease emergences in human populations are highlighted in green.

Supplementary Text 1: Animal Capture Methodology

For terrestrial mammals, two different field techniques were used on Madagascar. In montane humid forests, pitfall traps were used as previously described (1, 2). Briefly, a series of 12 l buckets, each separated by 10 m distance, are buried into the ground and with the rim flush with soil level. Each 100 m line commenced and terminated with a bucket. Bisecting each bucket was a continuous vertical plastic fencing, stapled to stakes and partially buried into the ground, which acted to impede the passage of small mammals and guide them towards the buckets. The second technique consisted of two types of live traps: Sherman (22,5 x 8,6 x 7,4 cm) (H. B. Sherman Traps Inc, Tallahassee, Florida) and National (39,2 x 12,3 x 12,3 cm) (Tomahawk Live Trap, Hazelhurst, Wisconsin), the former has a tendency to capture animals of less than 50 g and the latter animals of larger body size. Briefly, traps were baited with a suitable food source (peanut butter), and placed on the ground or in trees. Due to the diurnal nature of some of the studied species in Madagascar, traps were left *in situ* for six consecutive nights and visited twice per day, just after dawn and in the late afternoon, when bait was removed and replenished. These types of live traps were used for sampling on La Réunion, Mayotte and Mahé, including forested and more urbanized areas and where targeted species are nocturnal. Hence, traps were installed at dusk and inspected the following morning.

Bats were capturedat dusk or at night using mist nets or harp traps placed in proximity to cave entrances, across presumed flight pathways, or near water source where these animals come to drink at dusk. On several occasions, a butterfly hand net was used to collect bats inside their day roosts.

Captured animals were identified based on different external and cranio-dental characters. All animals were dispatched using humane techniques identified by professional mammalogist committees (3). Dissections were performed using sterile utensils. All tissue

samples were immediately conserved in liquid nitrogen, and stored at -80°C after transport to the laboratory.

64 **Supplementary Text 2: Animal Descriptions**

65 The family Tenrecidae is best considered endemic to Madagascar and composed of a
66 radiation belonging to the superorder Afrotheria, of African origin and comprising a diversity
67 of mammals (4). The Tenrecidae colonization of Madagascar and divergence from the
68 balance of Afrotheria is dated to the Middle Oligocene, about 30 Mya (5). These largely
69 animalivorous mammals are either terrestrial or arboreal and vary in body size from about 1
70 kg to a few g (6).

71 The second group is an Afro-Malagasy family of rodents, Nesomyidae of which the
72 Nesomyinae are endemic to Madagascar. The nesomyine radiation is dated to have diverged
73 during the early portion of the Miocene, about 20 Mya, and probably of African origin (5).
74 They are frugivores or granivores, either terrestrial or arboreal and vary in body mass from 1
75 kg down to about 20 g (6).

76 The last two groups involved in this study are both introduced to SWIO islands. The first
77 comprises shrews of the genus *Suncus* (family Soricidae). These animals can be human
78 commensals, as well as living in natural forest settings. Two species occur in the region and
79 at least one, *S. murinus*, was probably introduced by Arab traders between the 11^{th} to 14^{th}
80 centuries (7, 8). The second group includes rodents (family Muridae) and represented in the
81 region by two genera, *Rattus* and *Mus*. The earliest known physical remains indicate that
82 *Rattus* was first introduced to Madagascar also during the 11^{th} to 14^{th} centuries (9). In the
83 case of *Mus*, Malagasy populations are closely related to those from the Arabian Peninsula
84 and probably brought to the island during the Middle Ages (10).

85 In contrast to the Tenrecidae and Nesomyinae, each of which comprises a single
86 colonization by an ancestor that subsequently underwent an adaptive radiation, the bat fauna
87 of Madagascar and neighboring islands involves a considerable number of different
88 colonization events. For Madagascar alone, at least 24 different colonization events took
89 place, largely from African sources, although a few taxa appear to be Asiatic in origin (11).

The bat fauna of the island includes largely frugivorous or insectivorous species, Ranging in body mass from nearly 400 to 4 g (12).

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Animal species with co-infection (identification code)	Origin	Associated paramyxovirus L-gene Accession numbers
Microgale cowani (VS 1912)	Madagascar	KF245972, KF245973
Microgale cowani (VS 1989)	Madagascar	KF245983, KF245984
Microgale cowani (VS 2211)	Madagascar	KF246048, KF246049
Microgale dobsoni (VS 2215)	Madagascar	KF246051, KF246052

Supplementary Table S1: Paramyxovirus co-infections. Highlighted are animals from which multiple paramyxovirus L-gene sequences could be obtained, that showed clearly divergent sequences. Multiple discrete sequences are suggestive of viral co-infections.

137 **Host associations for the studied *Paramyxoviridae***

	Chiroptera	Rodentia	Scandentia/ Soricomorpha/ Afrosoricida
A. *Pneumovirinae*	***Eidolon helvum* (5)**: FJ971952-3, FJ971956-7, FJ971960	***Mus* sp. (4)**: AY729016, AY743909-10, NC_006579	-
B. *Rubulaviridae*	***Sturnira lilium* (2)**: EF095490, NC_009489	-	-
C. Unclassified Rubulavirus-Related	***Eidolon helvum* (6):** JX051319-20, HQ660092, FJ971943, FJ609195, HQ660109 ***Rousettus leschenaultii* (3)**: GU128080-2 ***Pteropus hypomelanus* (2)**: AF298895, NC004074 ***Pteropus* spp. (3)**: JX112711, AF326114, NC_007620 ***Rousettus aegypticus* (3)**: HQ660098, HQ660100, HQ660106	-	-
F. *Respiroviridae*	-	***Mus* sp. (10)**: AB005795-6, AB065186-9, AB195947-8, DQ219803, NC_001552	-
G.Henipaviridae and Unclassified Henipavirus-Related	***Eidolon helvum* (79)**: FJ609194, FJ971936-40, GQ168929, HE647821-2, HE647827, HE647829-34, HE801055-6, HQ660127, HQ660131-5, HQ660140, HQ660142, HQ660147, HQ660150, JN862567-70,JN862576, JN862578, JN862584-6, JN862588, JN862590, FJ609191, HE6478236, HE647828, HE647833, HQ660123-5, HQ660130, HQ660136, HQ660141, HQ660143-4, HQ660146, HQ660148-9, HQ660151, JN862562-6, JN862571-5, JN862577, JN862579-83, JN862587, JN862589, JN862591-4, FJ609198 ***Myonycteris torquata* (2)**: HQ660118, HQ660137 ***Hypsignathus monstrosus* (2)**: HQ660119, HQ660152 ***Epomorphorous* sp. (4)**: HQ660120-2, HQ660128 ***Rousettus aegypticus* (1)**: HQ660138 ***Pteropus* sp. (4)**: AB748559-61, JQ001776 ***Pteropus vampyrus* (1)**: FN869553 ***Myonycteris torquata* (1)**: HQ660126, ***Rousettus aegypticus* (2)**: HQ660139, HQ660145 ***Pteronotus parnellii* (1):** JF828297	***Rattus rattus* (1)**: AB844426	***Crocidura* spp. (9)**: AB844341, AB844343-50
I. Morbilliviridae	***Desdomonus rotundus* (2):** HQ660188-9	***Rhabdomys pumilio* (5)**: HQ660178-180, HQ660172-3 ***Apodemus* sp. (2)**: JF828302-3 **Mastomys natalensis (3)**: AB844407, AB AB844395, AB844382	
J. Unclassified Morbillivirus-Related	***Hipposideros* spp. (8)**: HQ660153-4, HQ660156-62 ***Coleura afra* (1)**: HQ660155 ***Triaenops menamena* (21)**: F928232, KF928233, KF928234, KF928235, KF928236, KF928237, KF928238, KF928239, KF928240, KF928241, KF928242, KF928243, KF928244, KF928245, KF928246, KF928247, KF928248, KF928261, KF928262, JQ886096, JQ886098 ***Miniopterus gleni* (1)**: JQ886097 ***Miniopterus griveaudi* (9)**: JQ886099-103, KF928257, KF928258, KF928259, KF928260 ***Miniopterus sororculus* (1)**: JQ886104 ***Mormopterus acetabulosus* (1)**: JQ886105 ***Pipistrellus* spp. (1)**: FJ609192, JN086951, JN086954 ***Hipposideros cafer* (1)**: HQ660158 ***Myotis spp.* (10)**: HQ660163-71, JN086953 ***Desdomonus rotundus* (1)**: HQ660187 ***Glossophaga soricina* (1)**: HQ660190 ***Carollia* spp. (4)**: HQ660191-4 ***Pteronotus parnellii* (2)**: JF828295-6 ***Chaerephon leucogaster* (1)**: KF928256 ***Mops leucostigma* (7)**: KF928249, KF928250, KF928251, KF928252, KF928253, KF928254, KF928265 ***Mormopterus jugularis* (2):** KF928225, KF928226 ***Myotis goudoti* (2)**: KF928227, KF928264 ***Otomops madagascariensis* (5)**: KF928228, KF928229, KF928230, KF928231, KF928263 **Vespertilionidae sp. (1)**: KF928255	***Microtus arvalis* (2):** HQ660184-5 ***Apodemus flavicolis* (3)**: JF828298, JF828308, HQ660183 ***Myodes glarious* (11)**: HQ660181-2, JF828299-301, JF828304-7, HQ660186 ***Mus musculus* (2):** NC_007454, AY900001 ***Rattus norvegicus* (13)**: DQ100461, KF245939-44, KF408257-60, AY286409, NC_005339 ***Rattus andamanensis* (1)**: JN689227 ***Rattus rattus* (32)**: KF245947-8, KF245950, KF245953-8, KF245960, KF246963, KF245976, KF245990, KF246003-4, KF246023, KF246025-7, KF246029, *KF245949*, KF245945, KF245961, KF245959, KF245951, KF246034, KF246002, KF246014, KF245968-9, KF246005, AB844427 ***Rhabdomys pumilio* (4)**: HQ660174, HQ660175-7 ***Mus minutoides* (2)**: AB844424-5 ***Mastomys natalensis* (15):** AB844369, AB844367, AB844401, AB844404, AB844376, AB844398, AB844406, AB844358, AB844388, AB844378, AB844391, AB844413, AB844396, AB844384, AB844422 ***Zygodontomys brevicauda* (2):** NC017937, FJ362497	***Suncus murinus* (2)**: KF245946, KF245952 ***Microgale* spp. (73)** : KF245962, KF245966-7, KF245970-5, KF245977-88, KF245991-7, KF246006-10, KF246015-21, KF246024, KF246030-1, KF246035-40, KF246042-56, KF246058-61, KF245964-5, KF246057, KF246041, KF246060 ***Eliurus* spp. (12)**: KF246011-2, KF246022, KF245998, KF246032-3, KF246013, KF245999-601, KF245989, KF246028 ***Tupaia belangeri* (1):** AF079780

138 **Supplementary Table S2:** Genbank accession numbers are presented for paramyxovirus sequences originating from bats,
139 rodents or other small mammal species that were of interest to this study. Sequences are grouped into the major clades of the
140 paramyxovirus family, based on family level phylogenies presented in main text Figure 2. Host-species names and the number
141 of sequences originating from that species are shown for each group. Sequences generated as part of this study are underlined.

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Country	Site	Latitude	Longitude	Altitude	Description	Capture Dates	Species Captured
Madagascar	Ambahibe	12°58.060'S	49°07.231'E	100 m	Dry deciduous forest	12 Sep. 2013	*Miniopterus griveaudi*
Madagascar	Andranoilovy	24°03'S	43°45'E	50 m	Spiny bush	21-26 Apr. 2012	*Triaenops menamena*
Madagascar	Androimpano	24°39.012'S	43°57.797'E	110 m	Spiny bush	23 Apr. 2012	*Triaenops menamena*
Madagascar	Anjohikinakina	19.0099°S	44.7677°E	130 m	Dry deciduous forest	6 Nov. 2012	*Myotis goudoti* *Otomops madagascariensis*
Madagascar	Ankarana National Park	12°55.9'S	49°03.4'E	50 m	Dry deciduous forest	15 Sep. 2012	*Triaenops menamena*
Madagascar	Ankazomivady	20.46494S	47.09444E	1675 m	Montane forest	9-14 Dec. 2010 3-8 Nov 2011 16 Mar. -3 Apr 2012	*Eliurus minor* *Rattus rattus* *Microgale cowani* *Microgale dobsoni* *Microgale gymnorhyncha* *Microgale longicaudata* *Microgale majori* *Microgale parvula* *Microgale principula* *Microgale taiva* *Hemicentetes nigriceps* *Setifer setosus* *Tenrec ecaudatus*
Madagascar	Antanandava	19.92793°S	44.60.594°E	40 m	Dry deciduous forest	2 May 2012	*Mops leucostigma*
Madagascar	Bekoaky	22°46'22.8"S	43°43'21.6"E	80 m	Dry deciduous forest	10 Feb. 2012	*Myotis goudoti*
Madagascar	Kirindy	20.06332°S	44.59679°E	50 m	Dry deciduous forest	1-2 May 2012	Vespertilionidae sp.
Madagascar	Lakato	19.0238S	48.2055E	1000 m	Lower Montane Forest	23-28 Oct. 2010 19-25 Mar. 2011 6 -21 Mar. 2012	*Eliurus minor* *Eliurus tanala* *Gymnuromys roberti* *Nesomys rufus* *Rattus rattus* *Rattus norvegicus* *Microgale dobsoni* *Microgale fotsifotsy* *Microgale jobihely* *Microgale longicaudata* *Microgale majori* *Microgale principula* *Microgale taiva* *Oryzorictes hova* *Suncus murinus* *Hemicentetes semispinosus* *Setifer setosus*
Madagascar	Grotte des Makis	23°28'19.6"S	43°46'14.5"E		Spiny bush	8 Feb. 2012	*Mormopterus jugularis*
Madagascar	Marofandilia	20°04.046'S	44° 39.480'E	20 m	Dry deciduous forest	1-2 May 2012	*Mops leucostigma*
Madagascar	Marofototra	20.30244°S	44.39807°E	20 m	Dry deciduous forest	4 May 2012	*Chaerephon leucogaster*
Madagascar	Tanambao	23°32.933'S	43°46.044'E	10 m	Spiny bush	10-12 Feb. 2012	*Otomops madagascariensis* *Triaenops menamena*
Madagascar	Tsimafana	19.92793°S	44.58432°E	40 m	Dry deciduous forest	2 May 2012 9 Nov. 2012	*Mops leucostigma*
Madagascar	Vintany	24°42'8.5"S	43°42'08.5'E	25 m	Spiny bush	24 Apr. 2012	*Triaenops menamena*
Mayotte (France)	Sada	12°52.680'S	45°07.339'E	70 m	Urban	12-18 Jul. 2012	*Rattus rattus*
Mayotte (France)	Combani	12°47.915'S	45°09.386'E	130 m	Forest	12-18 Jul. 2012	*Rattus rattus*
Mayotte (France)	Tzoundzou	12°48.349'S	45°12.261'E	20 m	Urban	12-18 Jul. 2012	*Rattus rattus*
Mayotte (France)	Convalesence	12°46.102'S	45°11.415'E	400 m	Forest	12-18 Jul. 2012	*Rattus rattus*
Mayotte (France)	Mbouzi	12°48.581'S	45°14.191'E	30 m	Forest	12-18 Jul. 2012	*Rattus rattus*
Mayotte (France)	Mamoudzou	12°46.778'S	45°13.671'E	10 m	Urban	12-18 Jul. 2012	*Rattus rattus*
Mahé (Seychelles)	Anse aux Pins	4°42.216'S	55°31.188'E	10 m	Residential	4-15 Jun. 2011	*Rattus rattus*
Mahé (Seychelles)	Zone 21	4°40.119'S	55°30.355'E	1 m	Residential, Coastal	4-15 Jun. 2011	*Rattus rattus*
Mahé (Seychelles)	La Misère	4°39.992'S	55°28.225'E	425 m	Dense Residential	4-15 Jun. 2011	*Rattus rattus*
Mahé (Seychelles)	Station forestière de Grande Anse	4°40.588'S	55°27.263'E	30 m	Secondary Forest	4-15 Jun. 2011	*Rattus rattus*
Mahé (Seychelles)	Helvetia Tea Plantation	4°40.446'S	55°27.214'E	75 m	Forest	4-15 Jun. 2011	*Rattus rattus*
La Réunion (France)	Diverse	-	-	-	-	Sep. 2012-Mar. 2013	*Rattus rattus* *Rattus norvegicus* *Suncus murinus* *Mus musculus*

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145 **Supplementary Table S3:** Description of capture sites, locations, dates and species captured.

Std / Mean	A	B	C	D	E	F	G	H	I	J
A	14.67 73.05	2.07	2.17	1.83	1.61	1.48	2.09	1.30	2.32	2.14
B	38.07	17.28 78.53	2.18	19.29	1.42	1.48	1.70	1.32	1.22	1.53
C	38.69	61.60	8.64 72.25	2.03	1.54	1.42	1.75	1.64	1.94	1.74
D	37.86	53.92	47.94	14.72 82.16	0.94	1.06	1.83	0.99	1.08	1.38
E	38,06	46.42	48.18	41.20	0.00 100.00	0.83	1.27	0.00	1.13	1.58
F	39,72	46.75	45.95	42.23	58.73	14.00 80.07	1.83	1.64	1.64	1.89
G	39.77	45.97	45.92	43.16	59.19	57.69	8.44 66.60	1.44	1.99	2.48
H	36.79	44.09	44.25	41.85	55.70	55.65	58.61	0.00 100.00	1.08	1.82
I	39.46	44.81	44.58	42.56	58.15	55.35	60.04	57.05	14.92 79.62	1.63
J	39.82	46.15	45.28	42.32	59.71	57.24	62.58	59.60	61.66	7,25 69.46

100,00
90,00
80,00
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00

A - *Pneumovirinae*, B - *Rubulaviridae*, C - Unclassified Rubulavirus-Related, D - *Avulaviridae*, E - *Ferlaviridae*, F - *Respiroviridae*
G - *Henipaviridae* and Henipa-Related viruses, H - Salem virus, I - *Morbilliviridae*, J - Unclassified Morbilli-Related viruses

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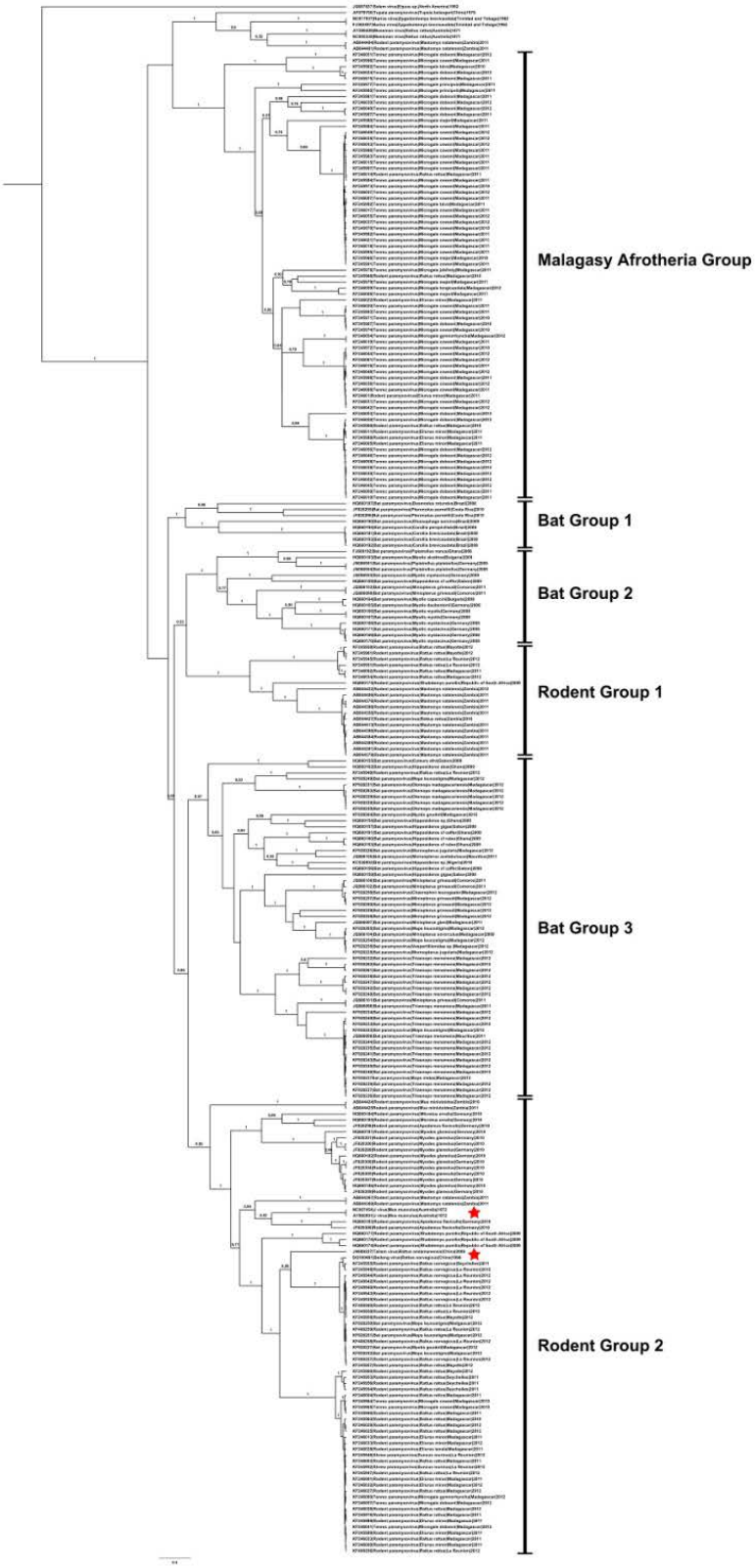
147 **Supplementary Figure S1:** Graphical representation of the genetic similarity (% identity) between different functional clade
148 groups of the *Paramyxoviridae* (see Figure 2). Numbers below the diagonal are the mean identities between all individual
149 members of the compared groups. Numbers above the diagonal are the corresponding standard deviations of sequence
150 identities. The indicated color scale of green to red highlights strong to weak sequence conservation.

151


Malagasy Afrotheria Group
Bat Group 1
Bat Group 2
Rodent Group 1
Bat Group 3
Rodent Group 2

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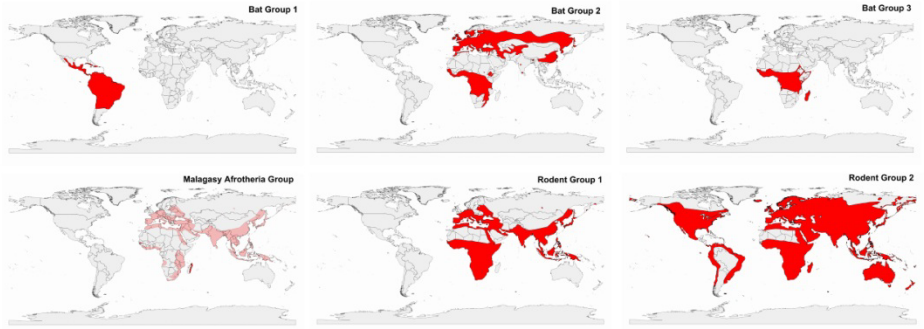
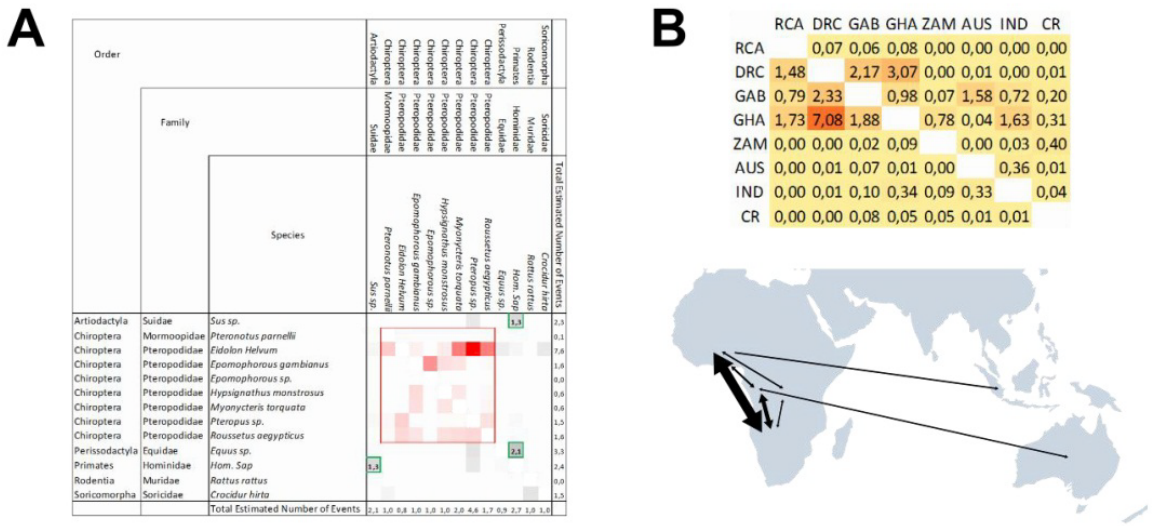
Supplementary Figure S2 (previous page). Phylogeny of all sequences belonging to the UMRV phylogroup. Groups are defined by majority host-species. Red stars indicate the positions of Beilong (DQ100461) and J-viruses (NC007454, AY900001) in the phylogenetic tree.



Supplementary Figure S3. Geographical distributions of paramyxovirus-positive species. The potential extent of each group presented in Supplementary Figure S2 is calculated from the geographical distribution of its hosts, according to the IUCN database (http://www.iucnredlist.org/), and is indicated in dark red to the right. Light red is used for locations outside of Madagascar for the "Malagasy Afrotheria Group", all sequences within this phylogenetic group originated from Madagascar including the minority of sequences that were detected in *R. rattus*. There is no evidence that viruses sharing sequence homology to the Malagasy Afrotheria Group exist outside of Madagascar.



Supplementary Figure S4. Ancestral state reconstructions – *Henipaviruses* and *Unclassified Henipa-related Viruses*. Ancestral host-species (A) and geographical (B) states were reconstructed and used to predict switching events in Mesquite based on 4000 raw trees of the *Henipavirus* phylogeny generated in BEAST. Host switching events that are linked with disease emergences in human populations are highlighted in green.

4.4. Conclusion du chapitre 4

Les analyses phylogénétiques sous BEAST nous ont permis tout d'abord de classer les paramyxovirus détectés dans un nouveau genre, proches des *Morbillivirus*, mais sans y être attaché. Nous les avons nommé «*Unclassified morbilivirus-related viruses* ou *UMRVs*». Une observation intéressante est le niveau de variabilité génétique très élevé de ces *UMRVs*. Nous avons également observé un niveau très élevé de diversité génétique au sein des *URMVs* des Chiroptera et des Rodentia. Par ailleurs, la diversité génétique estimée au sein des *URMVs* des Chiroptera est beaucoup plus importante en comparaison de celui estimé au sein des *URMVs* des Rodentia et Afrosoricidae démontrant le rôle des chauves-souris en tant que réservoir des paramyxovirus.

Une autre observation originale concerne l'étendue du spectre d'hôtes notamment par la mise en évidence de ces virus chez de nouvelles espèces, incluant les chauves-souris insectivores, tels que *Otomops madagascariensis*, les *Eliurus* (*E. minor*) et les microgales (*M. dobsoni, M. principula, M. majori, M. taiva, M. cowani, M. gymnorhyncha* et *M. johibely*). De plus, une certaine spécificité d'hôte a également été démontrée.

Un phénomène de saut d'espèce par lequel deux espèces d'hôtes phylogénétiquement très éloignées (par exemple appartenant à deux ordres d'animaux différents) hébergent des *UMRVs* phylogénétiquement extrêmement proches a été démontré. Cette caractéristique a été montrée entre les 3 ordres de mammifères étudiés.

De plus, nous avons montré que des paramyxovirus génétiquement similaires sont souvent repartis à travers le monde, suggérant la dissémination de ces virus sur de grandes distances (Aplin et al., 2003 ; Lund, 1994). Le rat, plus précisément *Rattus rattus*, est l'espèce qui serait la plus impliquée dans les processus de transmission de ces nouveaux virus, probablement par l'établissement de "pont épidémiologique" entre espèces animales très différentes.

Annexe 5 : Nos résultats semblent montrer que les *UMRVs* de Rodentia auraient pour origine l'Afrique et auraient disséminé vers le reste du monde *via Rattus rattus*. Cet article est en cours de soumission (Ghawar et al., soumis).

Chapitre 5. Les mécanismes moléculaires et les facteurs environnementaux associés à la diversité des Paramyxovirus chez les chauves-souris de Madagascar

5.1. Introduction

La compréhension des relations hôtes-pathogènes au niveau évolutif, ainsi que les interactions écologiques qui facilitent le maintien du pathogène dans son biotope naturel sont importants dans la prédiction des maladies émergentes (Morse et al., 2012 ; Childs et al., 2007).

Afin d'étudier les mécanismes macro-évolutifs prédominants chez les paramyxovirus de chauves-souris, nous avons conduit une série de campagnes de collecte au sein de consortia d'habitats naturels et anthropisés à Madagascar. Vingt-deux grottes, hébergeant soit une ou plusieurs colonies différentes de chauves-souris vivant en sympatrie pour certaines d'entre elles (voire même en syntonie), 18 habitats synanthropiques, et 12 sites forestiers, distribués sur toute la grande île, représentant un total de 947 individus qui ont été testés pour la présence ou non de paramyxovirus.

Les prévalences des paramyxovirus au niveau intra- et inter-hôtes, intra- et inter-grottes ont été estimées après criblage des individus par RT-PCR. Des analyses phylogénétiques sous Mr Bayes ont été réalisées pour déterminer l'histoire évolutive de ces virus. Des tests de co-phylogénies ont été effectués sous CoRe-PA pour l'identification du (ou des) mécanisme(s) principal (aux) macro-évolutif(s) chez les paramyxovirus.

Par ailleurs, une analyse statistique sous PaRaFit a été conduite pour mettre en évidence les éventuelles associations significatives entre les hôtes et les paramyxovirus. Enfin, identifier certains facteurs biotique et abiotique pouvant jouer un rôle dans la transmission de ces virus, nous avons réalisé une analyse statistique multivariée en passant par la construction modèle linéaire généralisé binomiale (GLM) sous R.

5.2. Etude éco-épidémiologique d'une communauté de chauves-souris de Madagascar : Analyses des processus macro-évolutifs et facteurs écologique associés à la transmission virale.

An eco-epidemiological study of Morbilli-related paramyxovirus infection in Madagascar bats reveals host-switching as the dominant macro-evolutionary mechanism.

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Abstract

An eco-epidemiological investigation was carried out on Madagascar bat communities to better understand the evolutionary mechanisms and environmental factors that affect virus transmission among bat species in closely related members of the genus *Morbillivirus*, currently referred to as *Unclassified Morbilli-related paramyxoviruses* (*UMRVs*). A total of 947 bats were investigated originating from 52 capture sites (22 caves, 18 buildings, and 12 outdoor sites) distributed over different bioclimatic zones of the island. Using RT-PCR targeting the L-polymerase gene of the Paramyxoviridae family, we found that 10.5% of sampled bats were infected, representing six out of seven families and 15 out of 31 species analyzed. Univariate analysis indicates that both abiotic and

biotic factors may promote viral infection. Using generalized linear modeling of *UMRV* infection overlaid on biotic and abiotic variables, we demonstrate that sympatric occurrence of bats is a major factor for virus transmission. Phylogenetic analyses revealed that all paramyxoviruses infecting Malagasy bats are *UMRVs* and showed little host specificity. Analyses using the maximum parsimony reconciliation tool CoRe-PA, indicate that host-switching, rather than co-speciation, is the dominant macro-evolutionary mechanism of *UMRVs* among Malagasy bats.

Introduction

The transgression of the species barrier by pathogens moving from their natural host reservoir to infect a new host species (also referred to as host-switching, host-jumping or host-shifting), may induce an abortive infection in the few infected individuals of the new host, or trigger a short lived outbreak, or an epidemic[1,2]. Co-speciation and host-switching are the two main evolutionary mechanisms generating genetic diversity among micro-organisms. Both are long-term dynamic processes[3], in contrast to co-evolution *sensu stricto*, which continuously acts on a short-time scale[4]. Co-speciation, the simultaneous speciation of the host and their parasites[3, 5-7], was considered for many years as the principal macro-evolutionary process generating viral diversity[8-11]. As convincing examples of co-speciation are rare, this mechanism has probably been overestimated. Host-switching refers to a new host-parasite combination that results from the shift of the parasite to a new host and its subsequent specialization, for example, under environmental selection pressure[12]. Colonization by a parasite of a phylogenetically closely related host species, often of the same genus or family, has proven to be the typical macro-evolutionary mechanism for RNA viruses[13]. An excellent example is the evolutionary history of *Hantaviruses* and *Arenaviruses*[14,15], mostly shaped by multiple host-jumps, followed by adaptive processes within the new host, as demonstrated in bats and other operative host species[16,17].

The often gregarious roosting behavior of bats and an assortment of different ecological parameters (e.g., climate, season, and migration) are important factors that can shape viral transmission dynamics, which subsequently act upon evolutionary processes[10,11,13,18]. Deciphering such mechanisms helps to understand how a virus hosted in wild animals can emerge as a pathogen in human populations[19]. For example, host-switching of *Ebola virus*, *SARS Coronavirus* or *Nipah virus* have led to major pandemics or epidemics in humans[2,20,21].

63 *Paramyxoviridae* is a large and diverse viral family (Order: Mononegavirales) composed of
64 single-stranded negative RNA viruses[22]. Newly recognized paramyxoviruses (PVs), named
65 *Unclassified Morbilli-Related Viruses* (*UMRVs*), have recently been shown to infect small mammals
66 around the world[23], such as bats and terrestrial small mammals from the southwestern Indian Ocean
67 (SWIO) islands[17,24], including the biodiversity hotspot of Madagascar[25]. The island is divided into
68 several unique bioclimatic zones, characterized by different meteorological regimes overlaid on
69 elevation and underlying geology[26], which in turn give rise to distinct vegetation types and highly
70 endemic biotic communities.
71 After rodents, bats (order Chiroptera), constitute the most abundant, diversified, and
72 geographically wide spread group of mammals in the world[27]. Genetic and fossil studies have
73 estimated the basal split of placental mammals in the superorder Laurasiatheria from their ancestors at
74 approximately 80-90 million years ago (Mya) and a diversification of bat families at approximately 62
75 Mya[28]. The Chiroptera of Madagascar are placed in eight different families and currently 45 species
76 recognized, of which 36 species (80%) are endemic[29-31]; it is assumed that most originated from
77 Africa. In certain cases, phylogenetic analyses provide evidence for recent periods of diversification.
78 For example, Malagasy *Miniopterus*, a notably speciose genus, colonized the island from an African
79 source population approximately between 4.5 and 2.5 Mya, followed by a second phase between 2.5
80 and 1 Mya[32].
81 An important characteristic of Malagasy bat communities is that species co-occupy day roost
82 sites in caves, buildings or tree cavities (often in forests) in different species combinations and varying
83 numbers. Furthermore, certain bat species may have indirect contact with other wild, introduced or
84 domestic animals, including synanthropic small mammals, which may imply contamination of shared
85 common water sources or fruits by bat urine/saliva[29]. Considering the notable species diversity and
86 high levels of endemism of Malagasy bats, as well as varying community structure and ecological
87 conditions in which they occur, Madagascar provides an excellent context to study virus transmission
88 in these animals. Herein, we examine the factors involved in interspecific transmission of *UMRVs* and
89 try to unravel the macro-evolutionary mechanisms underlying genetic diversification in these viruses.
90

Results

In total, 947 bats (867 insectivorous and 80 frugivorous), representing seven different families and 31 species, were collected at 52 sites in all six provinces of Madagascar: Antananarivo (n = 44 bats), Antsiranana (n = 125), Fianarantsoa (n = 178), Mahajanga (n = 207), Toamasina (n = 37), and Toliara (n = 356). The sampling sites included 22 different caves (n = 480 bats), 18 buildings (n = 290), and 12 different forested areas (n = 177). Thirty-one sites (n = 664 bats) contained at least two species and 21 sites (n = 283) were monospecific. The sampling sites were in different elevational zones, ranging from low (0 to 800 m, n = 40 sites), mid (801 to 1000 m, n = 6), and high (over 1000 m, n = 6), with 761, 101, and 85 bats collected in each zone, respectively. Seventeen sites were sampled in dry (n = 384 bats), 22 in sub-arid (n = 382), 11 in sub-humid (n = 144), and two in humid (n = 37) bioclimatic zones. Twenty-two sites (n = 377 bats) were visited during the summer (warm, wet) season and 30 sites (n = 570) during the winter (cool, dry) season.

Ninety-nine of 947 bats (10.5%) tested positive for PVs by RT-PCR, giving a global infection rate of 11.1% in insectivorous bats and 3.8% in frugivorous bats (df (degrees of freedom) = 1; n = 947; χ^2 $P = 0.02$). The infection rates varied according to province, from 4.5% in Antananarivo to 15.2% in Antsiranana (df = 5; n = 947; χ^2, $P = 0.01$). The infection rates of PVs for bats living in caves, buildings, and forests were 12.9%, 7.9%, and 7.9%, respectively (df = 2; n = 947 χ^2, $P = 0.041$). The fraction of sites hosting PV positive bats among the 31 multispecies sites and the 21 monospecific sites were 70.9% and 61.9%, respectively (df = 1; n = 947; χ^2, $P > 0.05$). The infection rates for PV were 11.4% in multispecies sites and 8.1% in monospecific sites (df = 1; n = 947; χ^2, $P > 0.05$). Infection rates at individual sites varied from 2.0% at ANJHB to 38.1% at VINT with no PV positive bat at 17 sites (n = 121) (see Figure 1 for identification of sites and associated acronyms). At low, middle, and high elevation, the fraction of sites hosting PV positive bats was 67.5%, 83.3%, and 50.0% (df = 2; n = 947; χ^2, $P > 0.05$), respectively, and the mean positive rates were 11.4%, 8.9%, and 3.5%, respectively (df = 2; n = 947; χ^2, $P > 0.05$). In the humid, sub-humid, sub-arid, and dry bioclimatic zones, the percentages of sites hosting PV positive bats were 50.0%, 54.5%, 72.7%, and 70.6%, respectively (df = 2; n = 947; χ^2, $P > 0.05$) and the mean positive rates were 5.4%, 6.3%, 12.0%, and 10.9%, respectively (df = 2; n = 947; χ^2, $P > 0.05$). PV positive rates were 7.9% and 12.1%

for bats captured during the summer and winter seasons, respectively (df = 1; n = 947; χ^2, $P = 0.038$). Sites with *UMRVs* detection rates higher than 20.0% are indicated on Figure 1.

Six of seven sampled bat families yielded PV positive individuals, with the exception being Hipposideridae, for which the only Malagasy species is *Hipposideros commersoni* (Table 1). The highest PV detection rate was in the family Rhinonycteridae (39.3%) and the lowest in the family Pteropodidae (3.8%) (df = 6; n = 947; χ^2, $P < 0.001$). Half of the sampled species (16/32) contained PV positive individuals. The highest PV infection rate was in *Triaenops menamena* (n = 21/42; 50.0%) and the lowest in *Miniopterus mahafaliensis* (4/89; 4.5%) (df = 31; n = 947; χ^2, $P < 0.0001$).Insectivorous species had significantly higher detection rates (96/867; 11.1%) than frugivorous species (3/80; 3.8%) (df = 1; n = 947; χ^2, $P = 0.02$). No significant difference was found associated with sex and age classes, regardless of diet, habitat or site (χ^2, $P > 0.05$).

Model construction procedure lead to a binomial Generalized Linear Model (GLM) explaining individual infection based on seven different effects (Table 2). Among abiotic factors, Mean Annual Temperature (MAT) had an overall effect where, Mean Annual Rainfall (MAR) did not show any overall relationship with infection. However, relationships between rainfall and infection appeared different across multi- *versus* single-species sites with a quadratic effect observed for MAR. Habitat type and the multispecies characteristics did not show any significant effects, but showed marginal interaction. The multispecies sites show higher infection rates, compared to monospecific sites, for caves compared to buildings and forest capture sites (Figure 2), reinforcing the important role of multispecies bat environments on PV infection. Diet was also associated with viral infection (Table 2), with higher infection among insectivorous bat species, whereas, age and sex did not show any significant relationships. Generalized Linear Mixed Model (GLMM) with species, locality, and province as random factors were tested separately and did not improve the fit, but models with family, species and locality failed to converge due to numerical issues in model estimation.

We conducted a Bayesian analysis on the PV sequences generated from positive Malagasy bats together with PV GenBank sequences from Madagascar and elsewhere in the world. All new PV sequences presented in this study were identified as *UMRVs*, as they appeared more closely related to Morbilliviruses[17] (Supplementary Figure S1), than to any other genera of the family *Paramyxoviridae*

family. The *UMRV*s were characterized by a high level of genetic variability and nucleotide sequences varied from 62.0 to 100% sequence identity. Only two sequence pairs of the 99 that tested positive were identical. Although *URMVs* showed weak exclusivity to their bat host species, two phylogenetic patterns were identified: (*i*) closely related *UMRV* sequences were hosted by bat species and families that are phylogenetically closely related, particularly those occupying day roost sites in the same caves i.e., *Miniopterus griveaudi* and *Myotis goudoti* at AMBB; *Miniopterus gleni* and *Miniopterus sororculus* at BEK; (highlighted in blue in Figure 3). This feature suggests that host-switching events might be favored by physical proximity between phylogenetically closely related bat taxa.

(*ii*) some degree of host-specificity for *URMVs* was found, with individuals of one host species having closely related *UMRVs*, independent of other individuals occurring at the same roost site, (i.e., *Triaenops menamena* at VINT TSP , and ANDRF2) highlighted in green, or distant sites, (i.e., *Triaenops menamena* at VINT and TSP), highlighted in red on Figure 3.

In some cases, a correlation was observed between the distance separating capture sites and the degree of nucleotide sequence similarity of the infecting PVs across sites. More specifically, conspecifics living on distant sites host *UMRVs* that display lower level of nucleotide similarity than those infecting bats at sites in closer geographical proximity (i.e., *Triaenops menamena* at VINT and TANA, *Miniopterus griveaudi* at AMBB and ANJHK1, and *Miniopterus griveaudi* at ANDFR and AMBB) highlighted in yellow in Figure 3. This suggests that increasing geographical distance favors virus genetic differentiation and/or low levels of virus migration between bat roosting sites.

Using CoRe-PA, we performed a consensus phylogram for both viruses and bats, presented along with their tanglegram depicting bat-virus associations (Figure 4A-B). By evaluating 5000 random cost schemes, CoRe-PA computed the most parsimonious reconstruction and predicted the frequencies for co-evolutionary events, including co-speciation, host-switching, duplication, and sorting. For the generated 24 OTUs subset (Table 3a), the best quality value obtained was 0.256 for a solution with eight co-speciation events, 21 duplications, 51 sortings, and 19 host-switches. For the 39 OTUs subset, CoRe-PA produced 57 reconstructions (Table 3b), with a quality value of 0.25 and five co-speciation events, 33 duplications, 57 sorting, and 24 host-switches. Hence, for both sets, no clear evidence of co-speciation between *UMRVs* and bat species was found. The statistical analysis suggests

fewer co-speciation events in the data set than expected by chance (99.0% of randomized data sets showed more than eight co-speciation events) but more host-switching events than expected (100% of randomized data sets showed less than 19 host-switching events) (Figure 5A-B). Thus, notwithstanding the numerous identified duplication and sorting events, host-switching events appear to be the predominant aspect in the evolutionary history in *UMRVs* identified from Malagasy bats, as compared to co-speciation.

We quantified the degree of congruence between bats and *UMRVs* topologies, and the potential individual associations for each of the two OTU subsets. The hypothesis associated with independent speciation events could not be rejected by ParaFit (ParaFitGlobal = 38.62571; $P = 0.067$), for the 24 OTUs subset, whereas a significant overall pattern of association (ParaFitGlobal = 46.158; $P = 0.002$) was calculated for the 39 OTUs subset. Eight of 50 (16.0%) individual host-virus links were significant, based on a $P < 0.05$ for the 90.0% threshold, and 19 out of 60 (31.7%) for the 98% threshold. Tables S3a and S3b summarize the different associations of *UMRVs* with their respective hosts and the corresponding *P*-values for the two OTU subsets. Among the different bat species, *Triaenops menamena* was the most coupled species for both OTUs subsets, and *Miniopterus mahafaliensis* for the 39 OTUs subset. Depending on nucleotide identity, we observed a discrepancy of the global association signal, which is related to specificity increasing genetic variability by increasing the number of clades (i.e., increasing the nucleotide acid identity between sequences).

Discussion

The overall *UMRV* infection rate in Malagasy bats was 10.5%, we also found that in some cases, that certain bat families or species showed higher PV detection rates-. Four bat species had particularly high *UMRV* infection rates: *Triaenops menamena*, *Mops leucostigma, Miniopterus grivecaudi*, and *Miniopterus gleni*. Except for the latter taxon, all were living at sites where substantial virus circulation was recorded (Figure 1). Whether these species have higher susceptibility to PV infection cannot be discerned based on current data.

Moreover, statistical modeling demonstrated that environments supporting multiple species are positively associated to viral transmission, with a marginal effect of natural habitats (caves) being

more prone to PV infection, whereas habitat type alone was not a significant predictor of infection. As previously reported, the spread of viruses between bat species is promoted by sympatric conditions, specifically multispecies day roost sites[10]. Other studies on bat rabies transmission demonstrated the importance of sympatric occurrence for viral infection[16]. The high detection rate in multispecies sites likely results from greater species diversity in caves[29], inducing a proximity effect between individuals, which has been previously shown to promote virus transmission. Further work correlating rates of infection in caves as a function of bat density would help support this hypothesis; however, because of seasonal variation in bat density associated with population cycling and possible dispersal movements, this aspect will be difficult to document based on field studies. Certain climatic factors also seem to promote viral transmission: the probability of PV infection increased at localities with higher mean annual temperature, which favors infection compared to cooler regions. This result supports the importance of warmer temperature on viral transmission[18]. Whereas PV infection showed no overall relationship with rainfall, average rainfall conditions favored PV infection in multispecies sites, compared to drier/wetter conditions and to single-species sites. Further analyses need to be conducted to have a greater understanding of the role of climatic factors on infection. Finally, we also note that circulation of *UMRVs* seems to be much more active among insectivorous than frugivorous bat species, with only 3.8% of the latter tested positive. These results confirm previous studies conducted on SWIO islands[17,24].

The bat-associated *UMRV* phylogeny underlines several points, particularly among the four taxa with the highest infection rates:

(i) bats collected sympatrically or some cases syntopically in the same day roost sites, for example, *Miniopterus grivecaudi* and *Myotis goudoti*, belong to the families Miniopteridae and Vespertilionidae, respectively, host closely related viruses, suggesting that host-switching events occurred between these species/families;

(ii) bat/virus co-phylogenies, suggest that co-speciation cannot explain the observed patterns. Host-switching is the predominant macro-evolutionary process. In either case, numerous reciprocal selection pressures that act over the short-term scale, such as the *sensu stricto* co-evolutionary process, also drive host-virus interactions. Indeed, micro-evolutionary aspects, including those implying

231 selection, drift, and dispersal, result in intraspecific co-divergence of viruses[33-36]. Using CoRe-PA, we
232 highlight the lack of congruence between bat and *UMRV* phylogenies. In previous studies it has been
233 shown that a large number of phylogenies, set at the family level, including the *Paramyxoviridae*, are
234 driven by this mode of macro-evolution[13]. Moreover, a considerable number of spillover events have
235 been reported between rodents and bats[17,23]. Our phylogenetic analyses show that the same *UMRVs*
236 infect different bat species or families, leading to the observed phylogenetic incongruence. This aspect
237 can, at least in part be explained by the extremely rapid evolution of some RNA viruses, which as a
238 consequence of their higher mutation rate[37] generate large quasi-species virus populations, allowing
239 for greater chances after a host-jump to adapt to a new host or, in other words, to promote a better
240 adapted variant that can be sustained in the new host[38]. Examples of such macro-evolutionary
241 processes, driven by host-switching, have been reported for *Puumala virus* and a *Hantavirus* detected
242 in bats from northern Europe and for which no evidence of co-divergence was observed[39]. This
243 scenario has also been cited for other Hantaviruses and is probably a general rule for this viral
244 family[14,40];
245 (iii) a viral allopatric process, in which a virus speciates within a host species living in different
246 geographical areas, and giving rise to independent evolution[12,13]. This may have occurred for
247 *Triaenops menamena*, *Mops leucostigma*, and *Miniopterus griveaudi*; these three taxa have relatively
248 broad distributions on Madagascar[26]. Interestingly, we could observe 7 major but phylogenetically
249 distant viral clades with 5 or more closely related viruses detected in different bat species or families.
250 This observation may suggest the circulation of 7 major *UMRVs* strain across Madagascar infecting a
251 large host range. The CoRe-PA analysis indicates for these three species, 33 events of duplication.
252 Duplication is a virus speciation event that occurs within the same host. This phenomenon can be the
253 consequence of events that affect only the host, i.e., adaptive co-evolution, such as environmental
254 adaptation. Such duplication events can be, for example, an immune pressure selection or virus
255 specialization related to adaptation to different organs of the host. We also disproportionately found
256 numerous duplication events in our analysis (21 duplications for the 24 OTUs and 33 duplications for
257 the 39 OTUs subsets). This was anticipated, as CoRe-PA tends to place too many nodes from the virus
258 tree near the root of the host tree. Consequently, whenever two descendant parasites (i.e., parasites that

emerged straight from the same ancestral parasite) share the same host, a duplication event is predicted.. One explanation could be associated with the capability of a virus within the host species to replicate independently. Different species of *Miniopterus* can be found roosting in strict syntopy and this close physical contact between related bat taxa may facilitate host-switching followed by mutation and duplication within the host. These sorting events might have multiple evolutionary causes and several hypotheses can explain these observations: (*a*) an ancient co-speciation event between the ancestor of the host and the virus, but the viral descendants subsequently went extinct; (*b*) an unidirectional and irreversible host-jump of the ancestral virus from one host to another; (*c*) no host-virus association ever existed between the virus and the respective sibling host;

(iv) as indicated by ParaFit Global test, significant associations were observed with the OTUs subsets (Tables S5a and S5b). We found significant linkage associations between species such as *Triaenops menamena* and both OTUs subsets. These results matched with our virus phylogeny analysis, which indicate some host-specificity, in particular for *Triaenops menamena*. We hypothesize that this association occurred after multiple host-switching events at some point in the past (macro-evolution) and continued in the form of a co-evolutionary adaptation (micro-evolution) inside the new host. Such phenomenon has also been reported in different Coronaviruses[41], for which co-evolution with bats seems to be the predominant evolutionary process. Since phylogenetically distant bat families hosted closely related *UMRVs*, the genetic distance between different groups of bats does not seem to be a major constraint for host-switching. Such results are important since host and virus traits determine the ability for a virus to infect a new host and host-switching events should *a priori* occur between phylogenetically closely related bat species on Madagascar. Besides, the occurrence of multiple interspecies transmissions, even to genetically distant host species, could be promoted by the existence of ubiquitous or alternative receptors for the virus[42,43]. It has been shown that genetic distances between bat species are a key factor for host-switching events[13,16,44]. However, our data also indicate that genetically closely related *UMRVs* infecting different bat species, sometimes occurring in geographically distant areas, may suggest the intervention of a probable vector, capable to connect these different populations[1]. Furthermore, except for regular bat foraging or dispersal movements, the black rat, *Rattus rattus*, introduced to Madagascar, has been identified as a significant reservoir of

287 *UMRVs*[17]. This rodent might be the ideal candidate to play this spreading role and establishing
288 epidemiological bridges between different species.
289
290 **Methods**
291 **Fieldwork and sampling.** This study used samples collected in the context of a long-term project to
292 document the land vertebrates of Madagascar based on voucher specimens and for a variety of
293 studies[26]. From February 2012 to March 2013, bats were captured in the six different provinces of
294 Madagascar using harp traps, hand nets, and mist nets. Some *Pteropus* fruit bats were purchased in
295 markets. Individual bats were identified to species using external and cranio-dental characters and
296 comparison to museum specimens. For each animal, different parameters, including age, sex, and
297 reproductive condition[45] were recorded and this information deposited in DRYAD
298 (http://www.datadryad.org/).
299 Bat tissue samples were collected in the field and immediately stored in liquid nitrogen, then
300 transferred to -80°C storage upon arrival in the laboratory. The geographic ranges of the captured bat
301 species were variable, with some having broad distributions nearly across the complete island and
302 others distinctly more restricted. Several species, especially insectivorous bats, occur sympatrically in
303 the same cave systems and in the same forest blocks, or synanthropically in human-built structures.
304 Information on the species, province and specific collection locality, sampling season, geographic
305 coordinates, elevation, habitat type, and the number of bat species found at each site and the associated
306 species composition are presented in Tables S1 and S2. Mean climatic conditions of the sampling sites
307 were extracted from the WorldClim database (http://www.worldclim.org/). We used the resolution
308 proposed by WorldClim as 30 arc seconds (~1km). An open-source GIS software, QGIS[46], was used to
309 generate the map for visualizing Madagascar bioclimatic regions proposed by Cornet[47].
310 **Ethics statement.** Animals used in this study were manipulated in strict accordance with the
311 guidelines for the handling of wild mammals[48]. All protocols strictly followed the terms of research
312 permits and regulations and were approved by licencing authorities: Direction du Système des Aires
313 Protégées et Direction Générale de l'Environnement et des Forêts and Madagascar National Parks

under different research permits (n°194/12/MEF/SG/DGF/DCB.SAP/SCB, 067/12/MEF/SG/DGF/DCB.SAP/SCBSE, and 032/12/MEF/SG/DGF/DCB.SAP/SCBSE). Animals were captured, manipulated, and dispatched with thoracic compression following procedures accepted by the scientific community for the handling of wild mammals[48]. *Pteropus* were purchased in a market and were not physically collected by the research team in a natural setting. Euthanasia was used for *Pteropus* and not any other bat genera. All fieldwork conducted on Madagascar was before the creation and implementation of an institutional and/or licensing committee on the island to issue such clearances. A CITES permit from the Malagasy national authority was issued for *Pteropus* tissue export (n°243C-EA06/MG12) to CRVOI on La Réunion.

Statistical procedures. Exploratory analyses were performed using Pearson chi-square (χ^2) or Fisher's exact tests in R software[49] (95% confidence intervals with continuity correction). With the intent of identifying variables potentially correlated with *UMRV* infection, we performed a binomial GLM analysis[50]. We first visually inspected the relationships between variables (mean annual temperature [MAT], mean annual rainfall [MAR], habitat type), and "multi" a binary factor indicating whether a given site contained multiple (> 2) or one species of bat. Graphic inspection suggested an overall effect of MAT and no effect of MAR. However, relationships suggested a linear interaction between MAR and habitat types, and a possible quadratic effect of rainfall within multi- *versus* single-species sites. Main and interaction effects were tested separately while accounting for the effects of other variables. We retained the best model according to Akaike Information Criterion (AIC). We then tested the effects of biotic variables (sex, diet, and age), on our best model, to determine a significance effect while accounting for the effects of abiotic factors. GLMM[51] were constructed with unbalanced variables (i.e., province, localities, and species - related to non-homogenous sampling) set as random factors in order to be compared to the best GLM fit. Statistical analyses were conducted with R software package[49].

Sample screening. Approximately 1 mm^3 of lung, kidney, and spleen collected from the same animal were pooled in DMEM medium and homogenized in a TissueLyser II (Qiagen, Hilden, Germany) for 2 min at 25 Hz using 3 mm tungsten beads. Total nucleic acids were extracted from the mixture

supernatant using the viral mini kit v2.0 and an EZ1 BioRobot (Qiagen). cDNA products were generated via reverse transcription (cDNA kit, Promega, Madison, Wisconsin, USA). PVs were detected by a semi-nested PCR targeting part of the L-gene polymerase gene, designed as to detect *Respirovirus*, *Morbillivirus*, and *Henipavirus* (RMH)[52]. The 400-600 bp PCR amplified cDNAs were purified using the Qiagen PCR purification kit and cloned into the pGEM-T vector system (Promega). Cloned PCR products were sequenced by the Sanger method (Big Dye sequencing kit, ABI, Genoscreen, Lille, France) using M13 standard sequencing primers.

Bioinformatics analysis. The sequences were first compared to the published sequences from the *Paramyxoviridae* and published *UMRVs* in GenBank (National Center for Biotechnology Information, Bethesda, Maryland, USA) online (www.ncbi.nih.gov) using BLASTn and BLASTx. The sequence quality of individual reads was assessed, and all sequences were processed using the Geneious Pro software package v7.1.8[53]. DNA sequences obtained from at least three independent bacterial clones were aligned to correct for most sequencing or PCR introduced errors. M13 Primer sequences were trimmed from the finalized sequences. The resulting partial sequences (~490 bp) of the L-gene polymerase gene were then aligned with Translation Alignment using the default ClustalW cost matrix in Geneious Pro software package. PVs sequences from bats reported in a previous study by Wilkinson et al.[17] were used for phylogenetic analysis (GenBank number to KF928225 to KF928256 and to K928261 to KF928265). PVs and bat Cytochrome b (Cyt-b) sequences used for the present analyses were deposited in GenBank under the reference numbers in respectively Table S3 and Table S4. Information concerning amplified PV sequences is given in Table S3. In order to classify the detected new paramyxoviruses, viral family-level phylogenetic analyses were performed. A total of 209 partial L-gene paramyxovirus sequences collected in Genbank were used. Sequences were trimmed to remove any free end gaps or were entirely removed if the obtained alignment did not provide at least 462 bp of non-gap overlap. Internal gaps were permitted. The tree was performed in 5,000,000 iterations in MrBayes with the GTR + G + I evolutionary model and a 10% burn-in rooted with an *Aquaparamyxovirus* sequence (GenBank number EF646380). Genbank accession numbers used for each virus genera are indicated in Table S6.

368 A best-fit nucleotide substitution model of the alignment was determined using jModelTest[54]
369 with the Corrected Akaike Information Criterion (AICc)[55], and the most appropriate one for *URMVs*
370 from Malagasy bats was GTR + G. Phylogenetic trees were constructed using MrBayes v3.2[56]
371 employing a Bayesian Markov Chain Monte Carlo (MCMC) method, rooted with a *Mumps* virus
372 sequence (GenBank number AY309060). A minimum of two independent runs were made, with four
373 chains in each run, for a total of 50 000 000, sampling every 5000 generations. The first 5000 trees
374 burn-in were discarded. The obtained effective sample size values (ESS) for each parameter were all
375 superior to 200. Trees obtained after the convergence point were summarized and visualized by
376 FigTree 1.4.2 (http://treebioedacuk/software/figtree).
377 Available full-length Cyt-b gene sequences corresponding to each bat species that were
378 positive for *UMRV* infection were downloaded from GenBank. When Cyt-b sequences were not
379 available for a given bat species, PCR using primers targeting the Cyt-b gene were performed[57] to
380 generate ~1140 bp sequences. All bat Cyt-b sequences were aligned, the GTR+I+G model was also
381 the most appropriate substitution model, and the phylogenetic relationship among bat species were
382 analyzed using RAxML 7.2.8 Geneious plugin[53] using 1000 generations. Two subsets of operational
383 taxonomic units (OTUs) were defined using Mothur[58], and based on two genetic distance cutoffs
384 (90.0% and 98.0%), generated 24 and 39 representative sequences, respectively.
385 To study the history of co-evolution of *UMRVs* with respect to their associated bat hosts, we
386 performed event-based co-phylogenetic reconciliation, using the tool CoRe-PA[59]. CoRe-PA is an
387 event-based maximum parsimony method, which attempts to construct the most parsimonious co-
388 evolutionary history of hosts and associated parasites. A cost is assigned to each type of co-
389 evolutionary event (co-speciation, host-switching, duplication, and sorting) and then, the parasite
390 phylogeny is mapped onto the host phylogeny, while trying to minimize the total costs of all occurring
391 events. In contrast to many other co-evolutionary software packages, CoRe-PA does not require an *a*
392 *priori* assignment of a cost scheme. It has been shown that the results of such analyses strongly depend
393 on the designed cost scheme, and choosing a biologically meaningful cost scheme in an *a priori*
394 *manner* may be difficult[59]. CoRe-PA can assess several random cost schemes and evaluate these,
395 based on the best fit with respect to the resulting reconstructions. In our study, we performed

reconstruction between the phylogenetic trees of *UMRVs* and bats, using 5000 random cost schemes. Furthermore, to test statistical significance, we computed the reconstructions of 100 random data sets, considering the same phylogenetic trees for bats and *UMRVs* with different bat and virus associations. In this case, the formulated null hypothesis is that there are more co-speciation and less host-switching events in the data set, than compared to data sets with random host-parasite associations.

We quantified the degree of congruence between bat and *UMRVs* topologies, and the potential individual associations leading to a potential co-phylogenetic structure using a global-fit method, ParaFit[60]. The latter program tests the independence of host and symbiont genetic or patristic distances, and specifically herein, tests the hypothesis of evolution independence between bats and *UMRVs*, i.e., one partner randomly evolving with respect to the other. Statistical analyses were done using the R software package[49].

Supplementary information

Table S1. Description of sampled sites in Madagascar.

Table S2. Number of species samples in each site.

Table S3. *UMRV* sequences data set.

Table S4. Bats cytochrome b sequences data set

Table S5. Test of host-parasite co-evolution using global fit methods ParaFit for the (a) 24 OTUs and (b) 39 OTUs.

Table S6. Paramyxovirus sequences data set.

Supplementary Figure S1. Phylogeny of all sequences belonging to the *UMRV* phylogroup.

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576 evolution mechanisms.

577 **Author contributions**

578 KD, SMG, and HP conceived and designed the study. JM performed the experiments. JM, NW, OF,
579 and HP analyzed the data. SMG, BR, YG, and EL conducted the bat field sampling and contributed
580 reagents/materials/analysis tools. JM, KD, SMG, and HP wrote the paper.

581 **Additional information**

582 **Supplementary information** accompanies this paper at http://www.nature.com/srep

583 **Competing financial interests:** The authors declare no competing financial interests.

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591

592 **Table 1. Detection rates of *UMRVs* in bats from Madagascar.** Numerator of individuals that tested
593 positive for PVs over total number of individuals tested, corresponding percentage of positivity given
594 in parentheses.

Family	Species	Total positive/total tested (%)	Grand total for family
Emballonuridae	*Coleura kibomalandy*	2/6 (33.3%)	2/9 (22.2)
	Paremballonura tiavato	0/3	
Hipposideridae	*Hipposideros commersoni*	0/27	0/27
Miniopteridae	*Miniopterus aelleni*	0/7	30/289 (10.4)
	Miniopterus cf. *ambohitrensis*	1/19 (5.3)	
	Miniopterus gleni	4/22 (18.2)	
	Miniopterus griffithsi	0/7	
	Miniopterus griveaudi	18/116 (15.5)	
	Miniopterus mahafaliensis	4/89 (4.5)	
	Miniopterus majori	0/7	
	Miniopterus sororculus	2/22 (9.1)	
Molossidae	*Chaerephon atsinanana*	0/34	36/406 (8.9)
	Chaerephon leucogaster	6/94 (6.4)	
	Mops leucostigma	11/68 (16.2)	
	Mops midas	1/19 (5.3)	
	Mormopterus jugularis	12/152 (7.9)	
	Otomops madagascariensis	7/39 (17.9)	
Pteropodidae	*Eidolon dupreanum*	0/11	3/80 (3.8)
	Pteropus rufus	3/20 (15.0)	
	Rousettus madagascariensis	0/49	
Rhinonycteridae	*Paratriaenops furculus*	1/14 (7.1)	22/56 (39.3)
	Triaenops menamena	21/42 (50.0)	
Vespertilionidae	*Hypsugo bemainty*	0/2	6/80 (7.5)
	Myotis goudoti	5/48 (10.4)	
	Neoromicia malagasyensis	0/2	
	Neoromicia matroka	0/4	
	Neoromicia robertsi	0/1	
	Pipistrellus cf. *hesperidus*	0/8	
	Pipistrellus hesperidus	1/11 (9.1)	
	Pipistrellus raceyi	0/3	
	Scotophilus marovaza	0/1	
Grand total			99/947 (10.5%)

595

596

597 **Table 2. Summary of the binomial GLM on individual infection (n=947).** The model was selected
598 after inspection of bivariate relationships and interactions. Because of unbalance, type III sums-of-
599 squares were used to test the effects. MAT: Mean Annual Temperature, Habitat: habitat type, Multi:
600 multispecies/monospecific site, MAR: mean annual rainfall, Df: degrees of freedom associated with
601 the effect, Deviance: deviance of the model, F value: value of Fisher statistics for the different effects,
602 Pr (>F): *P* values associated with the tests. Symbols for *P* values as follows: ^ < 0.1, * < 0.05, ** <
603 0.01, *** < 0.001.

Effect	Df	Deviance	F value	Pr (>F)
MAT	1	604.1	9.166	0.002**
Habitat	2	598.9	0.4938	0.61
Multi	1	605.7	11.65	0.0006***
Diet	1	602.6	6.803	0.009**
Habitat:Multi	2	601.3	2.423	0.0891^
Multi:MAR	2	604.6	5.006	0.007**
Multi:MAR^2	2	602.3	3.142	0.043*
Residuals		598.2		

604

605

606 **Table 3. Results for event base co-phylogeny obtained with CoRe-PA and number of the**
607 **different events for sets of (A) 29 OTUs and (B) 39 OTUs.** q indicates the quality values of each
608 reconstructions.

			Frequency of events				Total cost				
OTUs	Reconstruction	(q)	Co-speciation	Sorting	Duplication	Host-switch	Co-speciation	Sorting	Duplication	Host-switch	Total
A	1A	0.256	8	51	21	19	0.227	0.064	0.146	0.564	18.91
	2A	0.277	7	47	21	20	0.241	0.071	0.162	0.526	18.93
	3A	0.286	10	47	18	20	0.171	0.076	0.187	0.566	19.96
B	1B	0.25	5	57	33	21	0.22	0.07	0.13	0.58	21.52
	2B	0.26	5	51	32	22	0.23	0.08	0.13	0.56	21.84
	3B	0.28	6	63	33	20	0.15	0.05	0.08	0.7	21.1

609

610 **Figures Legends**

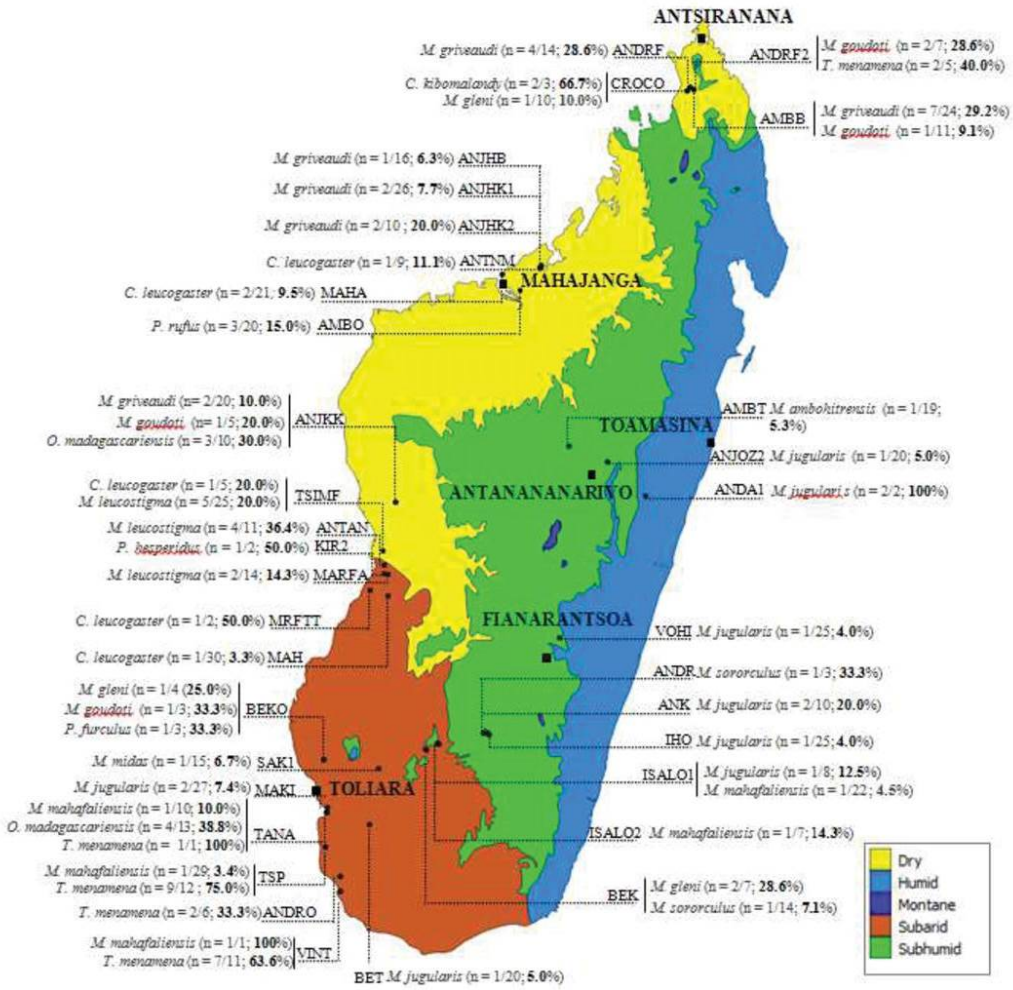
611 **Figure 1. PVs detection rates among the sites sampled on Madagascar.** Only sites containing
612 positive bats are represented. Abbreviations refer to the names of sampling sites (e.g. ANDRF for
613 "Andrafiabe"). n, numerator = the number of individuals that tested positive for PVs and denominator
614 = the number of individuals tested. Provincial capitals are indicated by black squares. QGIS[46], an
615 open-source GIS software (http://qgis.osgeo.org/en/site/), was used to generate the map for
616 visualizing bioclimatic regions of Madagascar proposed by Cornet[47].

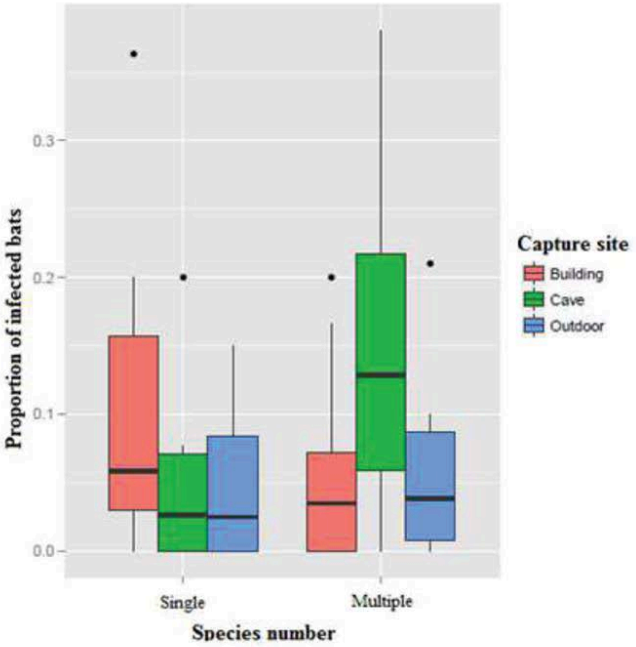
617 **Figure 2. Proportion of infected bats depending on species diversity at each sampling site and**
618 **the context of the where the samples were collected.** Individual outlying data points are displayed as
619 circles.

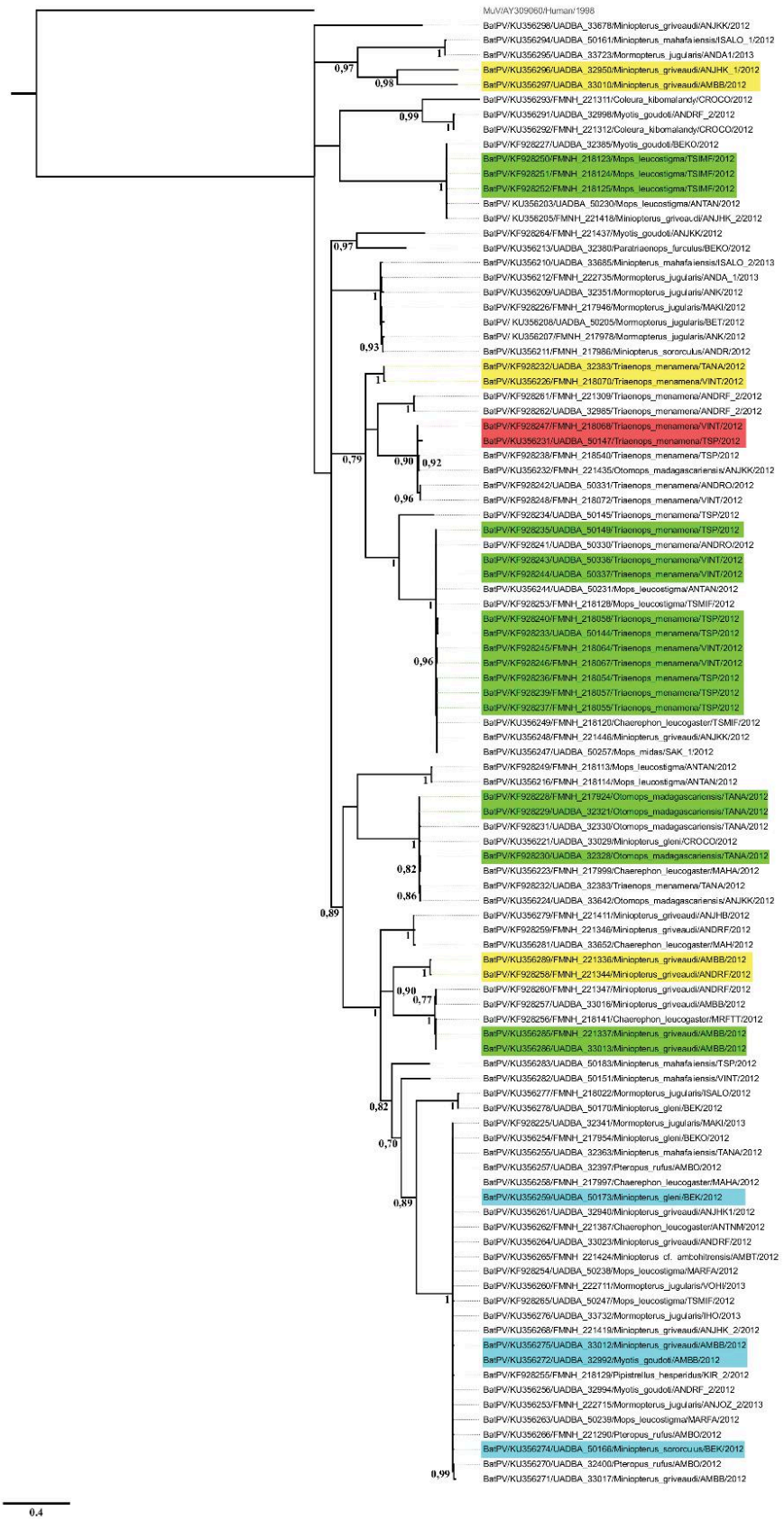
620 **Figure 3. Phylogeny of the *UMRVs* detected in bats from Madagascar.** A global phylogeny of 99
621 partial L-gene sequences calculated in 50,000,000 iterations in MrBayes with the GTR + G
622 evolutionary model and a 10% burn-in rooted with a *Mumps* virus sequence (GenBank number
623 AY309060). Only Bayesian with posterior probabilities > 0.7 were represented. Host switching events
624 were highlighted in blue and host-specificity for bats sharing the same sites in green. Bat species
625 occurring at distant sites are highlighted in red. Bats living at distant sites and hosting with low level
626 of *UMRVs* nucleotide similarity are highlighted in yellow.

627 **Figure 4. The first preferred reconstruction with the first best-cost model fit of the co-**
628 **evolutionary history for the set of (A) 24 OTUs and (B) 39 OTUs and associated bat-species**
629 **retrieved from CoRe-PA software.** Host tree is represented in black; parasite tree is represented in
630 grey.

631 **Figure 5. Frequency of co-speciation (A) and host-switches (B) events for the set of 24 OTUs**
632 **obtained by random reconstructions.** The number of co-speciation and host-switches events
633 expected in the most parsimonious reconstructions by CoRe-PA, 8 and 15, respectively (framed in red;
634 also in Table 3) were compared to the random reconstructions events below. The macro-evolutionary
635 events showing lower random reconstruction events than expected (8 or 15) was determined as the
636 predominant event.


ANTSIRANANA
M. griveaudi (n = 4/14; 28.6%) ANDRF
ANDRF2 M. goudoti (n = 2/7; 28.6%) T. menamena (n = 2/5; 40.0%)
C. kibomalandy (n = 2/3; 66.7%) M. gleni (n = 1/10; 10.0%) CROCO
AMBB M. griveaudi (n = 7/24; 29.2%) M. goudoti (n = 1/11; 9.1%)
M. griveaudi (n = 1/16; 6.3%) ANJHB
M. griveaudi (n = 2/26; 7.7%) ANJHK1
M. griveaudi (n = 2/10 ; 20.0%) ANJHK2
C. leucogaster (n = 1/9; 11.1%) ANTNM
MAHAJANGA
C. leucogaster (n = 2/21; 9.5%) MAHA
P. rufus (n = 3/20; 15.0%) AMBO
M. griveaudi (n= 2/20; 10.0%) M. goudoti (n= 1/5; 20.0%) O. madagascariensis (n = 3/10; 30.0%) ANJKK
AMBT M. ambohitrensis (n = 1/19; 5.3%)
TOAMASINA
ANJOZ2 M. jugularis (n = 1/20; 5.0%)
C. leucogaster (n = 1/5; 20.0%) M. leucostigma (n = 5/25; 20.0%) TSIMF
ANTANANANARIVO
ANDA1 M. jugularis (n = 2/2; 100%)
M. leucostigma (n = 4/11; 36.4%) ANTAN
P. hesperidus (n = 1/2; 50.0%) KIR2
M. leucostigma (n = 2/14; 14.3%) MARFA
C. leucogaster (n = 1/2; 50.0%) MRFTT
FIANARANTSOA
VOHI M. jugularis (n = 1/25; 4.0%)
C. leucogaster (n = 1/30; 3.3%) MAH
ANDR M. sororculus (n = 1/3; 33.3%)
M. gleni (n = 1/4 (25.0%) M. goudoti (n = 1/3; 33.3%) P. furculus (n = 1/3; 33.3%) BEKO
ANK M. jugularis (n = 2/10; 20.0%)
IHO M. jugularis (n = 1/25; 4.0%)
M. midas (n = 1/15; 6.7%) SAK1
ISALO1 M. jugularis (n = 1/8; 12.5%) M. mahafaliensis (n = 1/22; 4.5%)
M. jugularis (n = 2/27; 7.4%) MAKI
TOLIARA
M. mahafaliensis (n = 1/10; 10.0%) O. madagascariensis (n = 4/13; 38.8%) T. menamena (n = 1/1; 100%) TANA
ISALO2 M. mahafaliensis (n = 1/7; 14.3%)
M. mahafaliensis (n = 1/29; 3.4%) T. menamena (n = 9/12 ; 75.0%) TSP
BEK M. gleni (n = 2/7; 28.6%) M. sororculus (n = 1/14; 7.1%)
T. menamena (n = 2/6; 33.3%) ANDRO
M. mahafaliensis (n = 1/1; 100%) T. menamena (n = 7/11; 63.6%) VINT
BET M. jugularis (n = 1/20; 5.0%)
Dry
Humid
Montane
Subarid
Subhumid


Proportion of infected bats
0.3
0.2
0.1
0.0
Single
Multiple
Species number
Capture site
Building
Cave
Outdoor


MuVIAY309060/Human/1998
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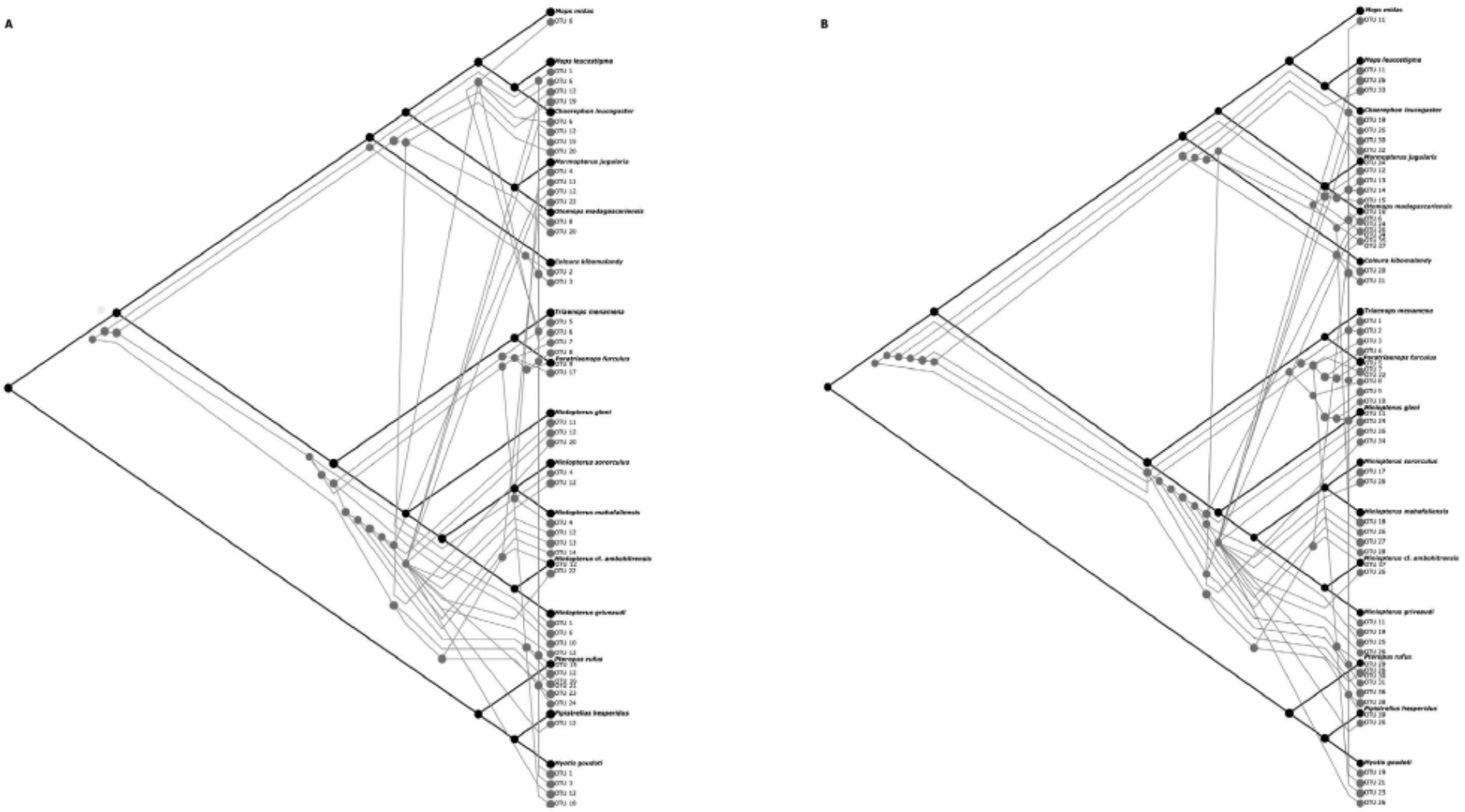
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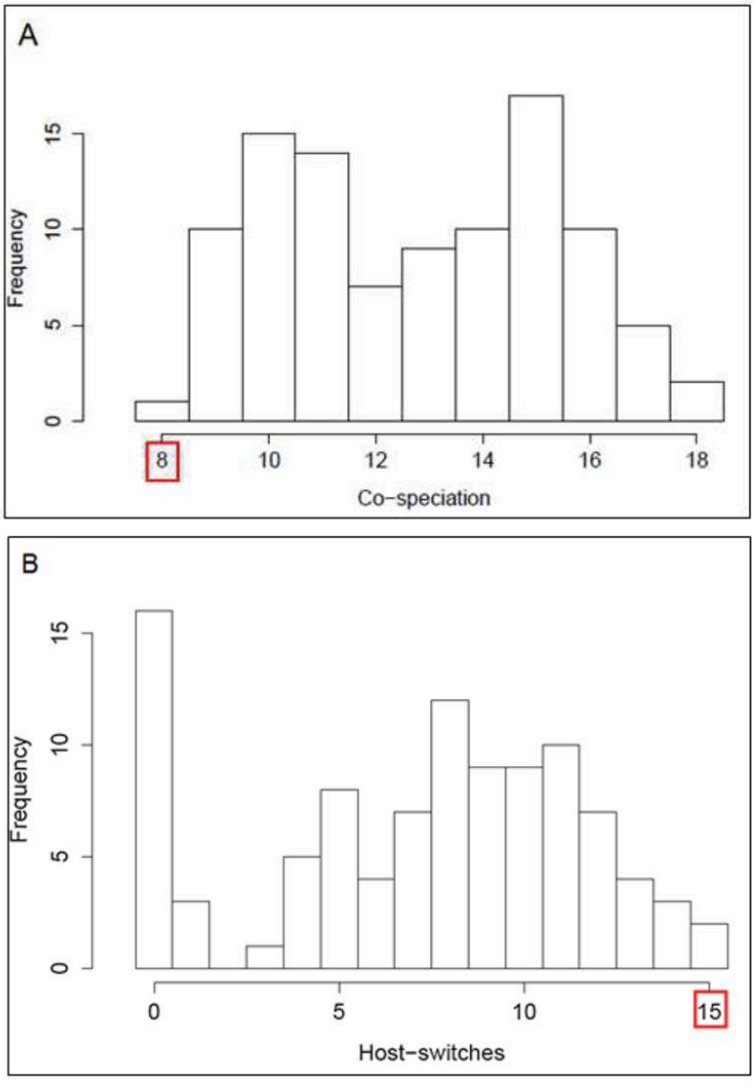
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5.3. Conclusion du chapitre 5

Nos résultats ont permis de caractériser de nouveaux Paramyxovirus chez une centaine de chauves-souris, appartenant à plus de 16 espèces différentes, et confirment la grande variabilité génétique de ces virus et l'étendue de leur spectre d'hôtes dans la faune sauvage.

L'analyse phylogénétique Bayesienne nous a conduit à classer les nouveaux membres de ces paramyxovirus dans le phylogroupe des *URMVs* permettant de dégager deux patrons phylogénétiques distincts:

(i) une spécificité d'hôte entre certaines chauves-souris géo-localisées au même endroit,

(ii) un saut d'espèces évident non seulement entre chauves-souris phylogénétiquement apparentées et physiquement proches, mais également entre chauves-souris phylogénétiquement et physiquement éloignées. Cette dernière observation suggère l'intervention probable d'une espèce intermédiaire jouant le rôle d'un "pont épidémiologique" entre chauves-souris qui ne sont *a priori* pas en contact.

Nous avons montré que le mécanisme macro-évolutif majeur mis en œuvre chez les *UMRVs* repose essentiellement sur le « *host-switch* », et non pas sur la co-spéciation. Ce mécanisme est sans doute à l'origine de la diversité génétique virale observée et du spectre d'hôte important.

Nous avons également caractérisé des associations statistiquement significatives entre certains hôtes et virus (spécificité d'hôtes) qui seraient le résultat, non pas de mécanismes macro-évolutifs (actifs sur le très long terme), mais probablement de mécanismes micro-évolutifs agissant sur des échelles de temps beaucoup plus courtes et en rapport avec l'adaptation de virus à son nouvel hôte postérieure au saut d'espèce.

L'analyse statistique multivariée a permis d'identifier 4 facteurs environnementaux qui favoriseraient soit une transmission virale, soit l'infection virale elle-même. Ils comprennent la température, le niveau de précipitations, un contexte multi-espèces, ainsi que le régime alimentaire insectivores. Le contact physique (proximité) entre chauves-souris dans les grottes (que ce soit entre espèces ou entre familles apparentées) influence la probabilité d'infection.

Ces travaux ont été valorisés sous forme d'un article scientifique soumis et d'une communication orale présentée au congrès international « **bats, small mammals and infectious agents** » à l'Université de La Réunion (2013).

Chapitre 6. Etude des lyssavirus chez les chauves-souris de la zone Sud-Ouest de l'Océan Indien

6.1. Généralités

La famille des *Rhabdoviridae* est composée de six genres : *Vesiculovirus*, *Ephemerovirus*, *Novirhabdovirus*, *Cytorhabdovirus*, *Nucleorhabdovirus* et *Lyssavirus*. Ce dernier genre comprend 15 virus (ICTV, 2014). Les 15 virus sont regroupés en 3 phylogroupes en fonction de leur antigénicité croisée et de leurs distances phylogénétiques (Badrane et al., 2001).

Les Lyssavirus sont retrouvés sur tous les continents à l'exception de l'Antarctique. La Figure en **annexe 3** illustre les différents réservoirs et la distribution géographique des différents variants de lyssavirus rapportés à ce jour. Les lyssavirus non rabiques ont été trouvés uniquement dans l'Ancien Monde alors qu'ils n'ont jamais été détectés en Amérique. A l'opposé, le continent américain est la seule partie du monde où il est courant de détecter le virus rabique (agent de la rage) chez les chauves-souris. Les seuls lyssavirus signalés en Afrique sont les virus Duvenhage, le virus de Lagos Bat, le virus Mokola, le virus Shimoni et le virus Ikoma.

Le virus rabique, agent de la rage, appartient au génotype 1 des lyssavirus, est l'espèce type du genre et aussi le plus important en termes de santé publique et vétérinaire. La rage est transmise à l'homme par la salive d'un animal infecté en phase d'excrétion virale. Le virus remonte à partir du point d'inoculation le long des terminaisons nerveuses, vers le système nerveux central. Quand ce dernier est atteint, le tableau clinique réalisé est celui d'une encéphalite aigue virale et à ce stade la rage est constamment mortelle (Tordo et al., 2010). Parmi les 14 autres lyssavirus, seuls EBLV-1 et 2, ABLV, DUVV, IRKV et MOKV ont causé des pathologies neurologiques similaires à la rage (Condori-Condori et al., 2013).

Le génome des lyssavirus est d'environ 12000 bases et code cinq gènes monocistroniques: une nucléoprotéine (N), une phosphoprotéine (P), une protéine de matrice (M), une

glycoprotéine (G) et une ARN polymérase ARN-dépendante (L). Les protéines N, P et L forment ensemble avec l'ARN, la nucléocapside (Lyles & Rupprecht, 2007).

Les protéines G forment des spicules qui se projettent à travers la membrane du virion. La protéine M relit la nucléocapside interne aux protéines G membranaires (Finke & Conzelmann, 2005) (**annexe 4**).

Le diagnostic de l'infection par les lyssavirus est basé i) soit sur la détection des antigènes viraux ou du génome viral (par RT-PCR ou qRT-PCR (Coertse et al., 2010) ou par isolement à partir de tissus infectés ou la salive ii) soit par la détection des anticorps spécifiques qui indique une exposition antérieure au virus. La technique « rapid fluorescent focus inhibition test » (RFFIT) détecte des anticorps neutralisants le virus rabique et est le test sérologique considéré comme le « gold standard ».

6.2. Approches expérimentales

Avant nos travaux, les investigations sur les lyssavirus de la région SOOI n'ont concerné que Madagascar, et encore, de façon très parcellaire. En effet, une seule étude antérieur décrit la présence d'anticorps neutralisants chez deux espèces de chauves-souris frugivores malgaches, *Rousettus madagascariensis* et *Eidolon helvum* (Reynes et al., 2012). La totalité des chauves-souris insectivores (6 espèces pour un total de 92 individus) analysées s'était révélée être séronégatives. L'étude régionale que nous rapportons dans mon travail de thèse apporte une information plus complète en démontrant la circulation intense des lyssavirus dans toutes les îles de la région.

Dans un premier temps, nous avons recherché la trace sérologique d'une infection passée des chauves-souris par des lyssavirus, en ayant recours à la technique RFFIT. Ce ne sont pas moins de 572 chauves-souris, représentant 22 espèces, qui ont pu être criblées pour la présence d'anticorps neutralisants contre 4 lyssavirus de référence: le virus rabique CVS-11, les lyssavirus DUVV, EBLV-1 et ABLV appartenant au phylogroupe 1 et le LBV appartenant au phylogroupe 2. Dans une seconde étape, le broyat de cerveau de chaque individu, à l'exception des chauves-souris de Mayotte, a été analysé par qRT-PCR pour rechercher la présence d'ARN viral. Cette étude sérologique a permis de démontrer la circulation des lyssavirus dans la faune sauvage de la région SOOI et d'établir le phylogroupe auquel ils appartiendraient.

Ces travaux ont été menés conjointement avec le laboratoire du Pr Wanda Markotter de l'Université de Pretoria en Afrique du Sud où j'ai réalisé un stage doctoral de 5 mois.

Annexe 6 : Dans un travail complémentaire, nous avons élargi l'enquête sérologique afin d'identifier les traces du passage de virus ARN simple brin dans la faune sauvage de la région SOOI. En utilisant la technique d'immunofluorescence indirect (ou Indirect Immunofluorescence assay IIFA) sur un panel de 15 virus de référence grâce aux lames de microscopie « Euroimmun Mosaic Chip » nous avons pu dresser le profil de réactivité sérologique à ces virus des populations de chauves-souris et de rongeurs de la région. Un total de 643 chauves-souris (29 espèces), de Madagascar, La Réunion, Maurice, Mahé, et Mayotte; ainsi que 199 rongeurs introduits (3 espèces) de Madagascar, Mahé, Mayotte and La Réunion et 57 petits mammifères endémiques de Madagascar (*Eliurus majori* et *Eliurus minor*) ont été analysés pour la présence d'anticorps dirigés contre en 5 familles virales décrites en annexe.

Cette étude nous a permis d'identifier au niveau de la famille virale et du genre, les agents infectieux viraux putatifs auxquels les animaux de la faune sauvage sont exposés. Bien que l'information ne puisse aller jusqu'à la caractérisation de l'agent viral lui-même, elle apporte une donnée précieuse sur la réactivité antigénique croisée entre les virus tests utilisés et les agents viraux putatifs, donnée utile pour l'identification des genres viraux en circulation, l'identification des principaux réservoirs en jeu, l'orientation des investigations complémentaires et l'évaluation du risque potentiel pour l'homme.

Ce travail a été mené conjointement avec le Laboratoire de Virologie du Pr Christian Drosten du Centre Hospitalier Universitaire de Bonn en Allemagne par la réalisation d'un stage doctoral de 3 semaines que j'ai effectué du 6 Septembre 2014 au 27 Septembre 2014.

6.3. Mise en évidence sérologique d'une circulation importante de lyssavirus chez les chauves-souris de la zone du Sud-ouest de l'Océan Indien.

PLOS ONE

Serological Evidence of Diverse Circulation of Lyssaviruses among Bats on Southwestern Indian Ocean Islands

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Article
Full Title:	Serological Evidence of Diverse Circulation of Lyssaviruses among Bats on Southwestern Indian Ocean Islands
Short Title:	Lyssavirus neutralizing antibody in bats from the Southwestern Indian Ocean Islands
Corresponding Author:	Julien Mélade Universite de la Reunion Ste Clotilde, RÉUNION
Keywords:	Lyssavirus, Bats, Rabies, South Western Indian Ocean, RFFIT
Abstract:	We provide serological evidence of diverse lyssavirus circulation among bats on southwestern Indian Ocean (SWIO) islands. A total of 572 bats, belonging to 22 species, from Anjouan and Mayotte (Comoros), Madagascar, Reunion Island, Mauritius, and Mahé (Seychelles) were screened by the Rapid Fluorescent Focus Inhibition test for the presence of neutralizing antibodies against five lyssaviruses representing both phylogroup I (Rabies virus, Duvenhage virus, European bat lyssavirus I, and Australian bat lyssavirus) and phylogroup II (Lagos bat virus). In total, 186 bats were able to neutralize at least one challenge virus, with large variation between islands, roost sites, and bat species. However, no lyssavirus RNA was detected by PCR of bat brains. These results highlight that lyssaviruses belonging to phylogroups I and II circulate in bat populations from the region, a feature that could be of public health importance, considering that certain bat species have contact with humans.
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Serological Evidence of Diverse Circulation of Lyssaviruses among Bats on Southwestern Indian Ocean Islands

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Abstract

We provide serological evidence of diverse lyssavirus circulation among bats on southwestern Indian Ocean (SWIO) islands. A total of 572 bats, belonging to 22 species, from Anjouan and Mayotte (Comoros), Madagascar, Reunion Island, Mauritius, and Mahé (Seychelles) were screened by the Rapid Fluorescent Focus Inhibition test for the presence of neutralizing

antibodies against five lyssaviruses representing both phylogroup I (*Rabies virus*, *Duvenhage virus*, *European bat lyssavirus I,* and *Australian bat lyssavirus*) and phylogroup II (*Lagos bat virus*). In total, 186 bats were able to neutralize at least one challenge virus, with large variation between islands, roost sites, and bat species. However, no lyssavirus RNA was detected by PCR of bat brains. These results highlight that lyssaviruses belonging to phylogroups I and II circulate in bat populations from the region, a feature that could be of public health importance, considering that certain bat species have contact with humans.

Introduction

Lyssaviruses (order: *Mononegavirales*, family: *Rhabdoviridae*) are RNA viruses with single-stranded, negative-sense genomes approximately 12 kb in length [1]. Fifteen distinct viral species, classified into three phylogroups, have been identified to date within the *Lyssaviruses* genus [2, 3]. These are the prototypic *Rabies virus* (RABV) and the 14 genetically related species referred to as rabies-related viruses that cluster into phylogroups according to their antigenicity reaction and phylogenetic distances [4]: *Duvenhage virus* (DUVV), *European bat Lyssavirus* types 1 (EBLV-1) and type 2 (EBLV-2), *Australian bat lyssavirus* (ABLV), *Irkut virus* (IRKV), *Aravan virus* (ARAV), *Khujand virus* (KHUV), and *Bokeloh bat lyssavirus* (BBLV) form phylogroup I. *Lagos bat virus* (LBV), *Mokola virus* (MOKV), and *Shimoni bat virus* (SHIV) form the phylogroup II. *Ikoma virus* (IKOV), *West Caucasian bat virus* (WCBV), and the newly described *Lleida Bat lyssavirus* (LLEBV) form the phylogroup III. With the exceptions of MOKV and IKOV, all lyssaviruses have been isolated from bats [5, 6].

RABV, the type species of the genus, is the principal causative agent of human rabies, a fatal acute encephalitis claiming 59 000 lives annually, primarily affecting Africa and Asia [7]. The natural cyclic maintenance of RABV worldwide is principally achieved by dogs and wild canids [8], while bats are seen as a reservoir only in the New World, where the virus is known to infect insectivorous and hematophagous species [9]. Nowadays, the majority of human exposure to rabies in the Americas is caused by contact with rabid bats [10]. In contrast, among the 14 rabies-related viruses, only EBLV-1 and 2, ABLV, DUVV, IRKV (phylogroup I), and MOKV (phylogroup II) have caused sporadic human deaths [9].

Islands of the southwestern Indian Ocean (SWIO) region, are seen as one of the world's biodiversity hotspots [11], hosting a rich chiropteran fauna. For example, on Madagascar with its 45 recognized bat species, 36 are endemic [12-14]; neighboring islands have notably lower measures of species diversity. Only one study conducted on Madagascar, a rabies endemic country where dogs are both maintenance reservoirs and vectors [15], has recorded antibodies to LBV and EBLV-1 in the frugivorous bat species *Eidolon dupreanum* and *Pteropus rufus*, but not six tested insectivorous bat species (n = 92) [16]. These antibodies are the trace indicators of past exposure in bats to a lyssavirus resulting in seroconversion. The other islands of the SWIO are deemed rabies free with no lyssavirus infection being recorded in non-volant mammals [15], although, the locally occurring bats have yet to be investigated. We report the results of a serosurvey conducted on bats from SWIO islands based on the screening of sera for lyssavirus neutralizing antibodies. This study is the first comprehensive analysis of SWIO region bats for exposure to lyssaviruses and aims to strengthen the available regional knowledge.

Methods

Specimen collection

From March 2010 to March 2015, 653 bats were collected from the islands of Anjouan and Mayotte (Comoros Archipelago), Reunion Island and Mauritius (Mascarene Archipelago), Madagascar, and Mahé (Seychelles Archipelago) (Figure 1), representing 22 species from 6 families. Both insectivorous and frugivorous bats were captured using harp traps, hand nets and mist nets. Some of the larger fruit bats were obtained from hunters. Individual bats were identified by morphological characters (outlined or cited [13]) and by

comparison to voucher specimens in The Field Museum of Natural History and The Département de Biologie Animale, Université d'Antananarivo.

Animals were manipulated in accordance with the guidelines for the handling of wild mammals [17], and all field protocols were designed to strictly follow the terms of the research permits issued by national authorities in the different countries this study took place (S1 Text). Details on ethical clearance are provided (S2 Text). This study has benefited from the sampling efforts conducted in the context of an ongoing international long-term project to catalog the terrestrial vertebrate fauna of Madagascar based on voucher specimens [18]. Bats trapped on Mayotte were released at the site of capture after sampling. Bat specimens sampled on the other islands where taken as voucher specimens housed in the above named museums. Details concerning the sex, the reproduction status [19], and age (wing bone ossification and dental eruption patterns) [20] for each captured bat were recorded. Names of capture sites were abbreviated (e.g. "Mangajou" abbreviate into MGJ) and reported in Table S.

Blood was collected by veinopuncture of the brachial vein or by cardiac puncture and subsequent exsanguinations (in some cases, needle and syringe were rinsed with heparin or EDTA to avoid blood coagulation during the procedure). Sera were separated from blood by centrifugation at 5000 rpm for 5 minutes. Brain was also collected from each of the vouchered individuals. Tissue samples were immediately stored in liquid nitrogen in the field and then transferred to -80°C freezers upon arrival at the laboratory.

Detection of lyssavirus neutralizing antibodies

Detection and titration of lyssavirus neutralizing antibodies were performed using the miniaturized Rapid Fluorescent Focus Inhibition Test (RFFIT) [21]. The challenge viruses used in the RFFIT were selected to represent two of the three phylogroups of lyssaviruses [3]:

RABV (CVS-11), DUVV (isolate DUVVSA06), EBLV-1 (isolate RV20), ABLV (isolate RV634), as representatives of phylogroup I, while LBV (isolate LBVAFR1999 Africa 1999) represented phylogroup II. Sera were tested at 1:5, 1:25, 1:125, and 1:625 dilutions. Each neutralization assay was performed in triplicate and was read by two different individuals. Serum samples producing neutralization above the threshold value of 1:5 with > 50% reduction of fluorescent foci were considered positive. Of the 653 serum samples, 81 were excluded from analysis either because they displayed a cell-adhesion inhibitory-type cytotoxicity (n = 45) or because the volume of sera was insufficient to do the assay in triplicate (n = 36). Hence, RFFIT was performed on 572 samples: all of which were tested against LBV as the phylogroup II challenge virus; 528 were also tested against CVS-11 and 540 also screened against DUVV as phylogroup I representatives. A subset of 220 samples had enough serum to allow additional testing against EBLV-1, and only 14 samples were screened for the presence of ABLV neutralizing antibodies.

Lyssaviruses RNA Detection

For 550 bats, total RNA was extracted from brain with Trizol (Invitrogen, Carlsbad, California, USA). Lyssavirus RNA detection was performed using a real time reverse transcription PCR (QuantiTect Probe RT-PCR Kit, QIAGEN, Cat number 204443) with specific primers targeting the conserved region of the nucleoprotein genes of lyssaviruses [22].

Statistical analysis

Statistical analyses were performed using Pearson chi-square (χ^2) or Fisher's exact tests in R software for calculation procedures (95% confidence intervals with a continuity

121 correction). Probability values < 0.05 were considered statistically significant. All statistical
122 computations were conducted using R version 3.0.0 (RCoreTeam, 2013).

123 **Results**

124 Of the 653 serum samples collected, we were able to screen 572 samples by RFFIT for
125 neutralizing antibodies to bat associated lyssaviruses, namely CVS-11, DUVV, EBLV, and
126 ABLV all belonging to phylogroup I and LBV from phylogroup II. These samples were from
127 421 insectivorous and 151 frugivorous bats, belonging to six bat families and 22 species. We
128 detected 186 individuals (32.5%) capable of neutralizing at least one of the challenge
129 lyssaviruses above the 1:5 threshold. Seropositivity rates differed significantly between
130 islands: being highest on Reunion Island, Mauritius, and Mahé, with 42.9% (n = 52/121)
131 41.8% (n = 28/67), and 32.5% (n = 13/40,) respectively, while on Madagascar, Mayotte, and
132 Anjouan, the seropositivity rates dropped to 27.9% (n = 84/301), 22.7% (n = 5/22), and 19.0%
133 (n = 4/21), respectively (p = 0.01) (Table 1). The overall proportion of seropositive
134 individuals between females (n = 125/338; 37.0%) and males (n = 61/234; 26.1%) was
135 different (p = 0.005). The seropositivity rates did not significantly differ between
136 insectivorous bats (n = 134/421; 31.8%) or frugivorous bats (n = 52/151; 34.4%) nor between
137 neonatal (n = 0/2; 0.0%), juvenile (n = 16/42; 38.1%), sub-adult (n = 17/75; 22.7%), and adult
138 specimens (n = 153/453; 33.8%).

139 All six bat families represented in the samples had seropositive individuals to at least
140 one of the challenge viruses. At the taxon-level, 12 out of the 17 insectivorous bat species
141 tested seropositive to at least one of the challenge viruses. A significant difference (p < 0.001)
142 between species was observed with *Hipposideros commersoni* (Hipposideridae), and
143 *Mormopterus francoismoutoui* and *M. acetabulosus* (Molossidae) displaying the highest

144 seropositivity rates with 50.0% (n = 3/6), 42.9% (n = 52/121), and 38.7% (n = 12/31),
145 respectively. Among the Molossidae, each of the three sister species of *Mormopterus*, *M.*
146 *acetabulosus*, *M. francoismoutoui*, and *M. jugularis* [23] endemic to Reunion Island,
147 Mauritius, and Madagascar, respectively, had a high proportion of seropositive individuals --
148 42.9% (n = 52/121), 38.7% (n = 12/31), and 34.5% (n = 20/58), respectively. Two of the three
149 frugivorous bats endemic to Madagascar (*Pteropus rufus* and *Rousettus madagascariensis*)
150 tested positive (n = 8/12; 66.7% and n = 12/35; 34.3%, respectively), while the third, *Eidolon*
151 *dupreanum*, tested negative (n = 0/9). Frugivorous species found on other regional islands
152 were also positive: *P. niger* on Mauritius (n = 16/36; 44.4%), *P. seychellensis* from Mayotte
153 (n = 3/19; 15.8%), and *P. seychellensis* Mahé (Seychelles) (n = 13/40; 32.5%). Six bat
154 species, all from Madagascar, tested seronegative against all challenge viruses: *Chaerephon*
155 *atsinanana* (n = 16), *Miniopterus mahafaliensis* (n = 11), *Eidolon dupreanum* (n = 9), *Mops*
156 *midas* (n = 7), *Miniopterus gleni* (n = 4), and *M. sororculus* (n = 3).

157 Overall, and for both frugivorous and insectivorous bats, antibodies neutralizing
158 DUVV were shown to be the most common: 97 sera out of 540 (17.9%) neutralized DUVV
159 only and 13 out of 220 (5.9%) neutralized EBLV-1 only. Besides this, 42 samples of 572
160 (7.3%) neutralized exclusively LBV, indicating past exposure to a phylogroup II virus. Many
161 sera reacted with more than one virus representative of phylogroup I: 32 of 220 (14.5%)
162 simultaneously neutralized DUVV and EBLV-1; one sera of 14 (7.1%) neutralized CVS-11
163 and ABLV, and 1 sera of 218 (0.5%) cross-neutralized CVS-11 and EBLV-1. No serum could
164 neutralize both challenge viruses of phylogroups I and II. In Table 1 we present the reactivity
165 profile with all challenge viruses according to island and bat family and species sampled.

166 There was no apparent specificity of bat species to one challenge lyssavirus: all bat
167 species, contained individuals that could neutralize either of the three test viruses, with LBV

168 being constantly the least reactive. Four exceptions were observed: *Miniopterus* cf.
169 *ambohitrensis* (Miniopteridae) reacted only with DUVV, *Mops leucostigma* (Molossidae),
170 *Myotis goudoti* (Vespertilionidae), and *Triaenops menamena* (Rhinonycteridae) reacted only
171 with LBV.

172 Looking at intra-island variation, several patterns emerge. On Madagascar, significant
173 difference were recorded between four bioclimate zones with the highest rate recorded in the
174 sub-humid region (n = 21/58; 36.2%) ($p < 0.001$) and among the low, middle, and high
175 elevational zones with 27.9% (n = 64/229), 11.5% (n = 3/26), and 36.9% (n = 17/46),
176 respectively, with the highest rates at high elevation ($p = 0.05$). Significant difference in
177 seroprevalence rates for positive animals was recorded between species, with the highest rate
178 in *Pteropus rufus* (n = 8/12; 66.7%) and the lowest in *Mops leucostigma* (n = 2/20; 10.0%).
179 Significant difference was also observed among localities with higher rate in AMBO (n =
180 8/12; 66.6%), the site the *P. rufus* were obtained and lower in AMBB (n = 1/11; 9.1%).
181 Twelve localities out of 33 were negative to the four challenge viruses (n = 70). On
182 Madagascar, seropositivity rates were statistically not different at sites containing multiple
183 species (n = 51/190; 26.8%) as compared to sites with single species (n = 33/111; 29.7%) or
184 between bat populations collected in caves (n = 46/158; 29.1%), in synanthropic roost sites (n
185 = 29/109; 26.6%), and in forests (n = 9/34; 26.5%). Rates were similar between insectivorous
186 bats (n = 64/245; 26.1%) and frugivorous bats (n = 20/56; 35.7%). No statistically significant
187 difference was recorded in seroprevalence rates in bats belonging to a given species collected
188 at different localities, whether captured at sites with multiple or single species (i.e.,
189 *Miniopterus griveaudi* at sites AMBB, ANDRF, ANJHB, ANJHK1, and ANJKK). Moreover,
190 seroprevalence rates were not significantly different in bats common to some islands, such as
191 *P. seychellensis* on Mayotte (n = 3/19; 15.8%) and on Mahé (n = 13/40; 32.5%). On Reunion

192 Island, seropositivity rates were higher in females (n = 46/90; 51.1%) than males (6/31;
193 19.4%) ($p = 0.001$) and significantly higher seroprevalence rates was observed in bats
194 captured in a maternity cave (50/100; 50.0%) as compared to those from a synanthropic
195 locality (n = 2/21; 9.5%) ($p < 0.001$). In contrast, on Mahé, seroprevalence rates in *P.*
196 *seychellensis* were significantly higher in male bats (n = 10/21; 47.6%) as compared to
197 females (n = 3/19; 15.8%) ($p = 0.03$). No other significant differences were recorded between
198 islands.

199 Various patterns of lyssavirus antibodies detection were observed on the different
200 regional islands (Figure S1-S6). In most cases, positive sites did not show specific patterns.
201 There were, however, some exceptions. For example, on Mayotte, where four positive sites
202 were detected, bats neutralized DUVV only at two sites (n = 2/15; 13.3%) and LBV only at
203 two other sites (n = 3/7; 42.9%). On Reunion Island, one site contained bats that neutralized
204 only DUVV (n = 2/21; 9.5%) and the other one combined animals that neutralized LBV or
205 cross neutralized DUVV and EBLV-1 (n = 50/100; 50.0%). The same feature was observed
206 on Anjouan, where one locality contained bats that neutralized only DUVV (n = 1/6; 16.7%)
207 and the other one combined animals that neutralized DUVV or LBV (n = 3/13; 23.1%). On
208 Mahé, the two positive localities combined animals that neutralized DUVV or LBV, cross
209 neutralized DUVV and EBLV-1 or cross neutralized CVS-11 and EBLV-1 (n = 13/40;
210 32.5%). On Mauritius, where seven positive sites where detected, bats neutralized DUVV
211 only (n = 5/14; 35.7%) at three localities and the four other positive localities combined
212 animals that neutralized DUVV, EBLV-1 or LBV or cross neutralized DUVV and EBLV-1
213 lyssaviruses (n = 23/53; 43.4%). On Madagascar, bats neutralized DUVV only (n = 31/105;
214 29.5%) or LBV only (n = 5/29; 17.2%) at 11 and three localities, respectively. The seven

215 other positive localities combined animals that neutralized DUVV, EBLV-1 or LBV, cross
216 neutralized DUVV and EBLV or cross neutralized CVS-11 and ABLV (n = 48/97; 49.5%).

217 No lyssavirus RNAs were detected by PCR in the nucleic acids extracted from the
218 brains of 550 different bats, whether seronegative or seropositive, with either of the test
219 lyssaviruses.

220 **Discussion**

221 Although several studies have reported on seroprevalence to lyssaviruses in bats in
222 Africa and Asia [6, 24-30], limited information is available for the SWIO islands, which are
223 located between these two continents. Our study reveals serological evidence of wide spread
224 lyssavirus exposure, often at high prevalence rates, among frugivorous and insectivorous bats,
225 on all investigated SWIO islands. At a regional scale, our study considerably expands the
226 geographic coverage and bat species investigated when compared to the single study
227 previously published on two Malagasy fruit bats, *Eidolon dupreanum* and *Pteropus rufus*
228 [15]. Seroprevalence rates reported herein are similar to those reported in Africa, northern
229 Vietnam, Cambodia, and the Philippines [24, 31-33]. Considering the close genetic
230 relationships between lyssaviruses belonging to the same phylogroup, the dual neutralization
231 occasionally observed between DUVV and EBLV-1 was expected [4, 25]. For those sera that
232 reacted with one single challenge virus of phylogroup 1, one should consider the possibility
233 that cross-neutralizing antibodies may have disappeared at the time of testing, long after
234 infection of the bat by lyssavirus.

235 Our data highlight that 12 species of insectivorous bats and almost all species of
236 frugivorous bats from the SWIO region, are seropositive towards at least one lyssavirus
237 species. Particularly high rates of prevalence exposure was found in *Hipposideros*

238 *commersoni* (Madagascar), *Mormopterus francoismoutoui* (Reunion Island), and *M.*
239 *acetabulosus* (Mauritius). Each of these three species contained high neutralizing antibodies
240 rates to DUVV or DUVV and EBVL-1. In agreement with earlier reports [32] showing higher
241 rates in females, particularly in nursing colonies [33], we were also able to demonstrate higher
242 seroprevalence rates in a maternity cave on Reunion Island, where 89 out of the 100 captured
243 individuals were females. In a previous study [34], it has been shown that this maternity
244 colony on Reunion Island, which harbors tens of thousands of female bats and their offspring,
245 is a hotspot for pathogen transmission between roosting individuals. Longitudinal studies are
246 warranted to analyze the temporal dynamics of antibody responses to lyssaviruses in bats
247 living in stable or intermittent roosting sites, such as maternity colonies. Environmental
248 conditions have been documented to promote exposure to and circulation of bat lyssaviruses
249 [35]. This was also apparent in our study where higher seroprevalence rates were detected in
250 sub-humid region and at higher elevations. However, we did not observe any significant
251 difference in seroprevalences according to age classes of the sampled bats.

252 Based on the sero-neutralization profiles, at least one circulating lyssavirus should
253 belong to phylogroup I, since most of positive sera neutralize DUVV or EBLV-1 or cross
254 neutralize CVS-11 and ABLV or EBLV-1. Interestingly, at the regional level, if we exclude
255 data obtained with ABLV (because of the limited number of samples tested with this virus),
256 the highest rates of seroreactivity are related to DUVV (17.9%), followed by a dual reactivity
257 with DUVV and EBLV (14.5%), then only with LBV (7.3%) and this reactivity profile is
258 consistent across each island. In contrast to previous studies on African bats [36, 24], where
259 antibodies to DUVV were mainly detected in insectivorous bats and antibodies to LBV in
260 frugivorous bats, our data reveal that antibodies to DUVV were detected at high rates, in both
261 these two groups of bats on SWIO islands. The very low reactivity of sera towards CVS-11

262 strengthens the hypothesis of a closer phylogenetic relationship between the actually
263 circulating virus with DUVV or EBLV-1 than with CVS-11.

264 Our results also support the view that a phylogroup II lyssavirus, closely related to, if
265 not LBV itself, is circulating in the region. This is in agreement with the study of Reynes and
266 colleagues [16], who reported that 24% of sera from Malagasy *Eidolon dupreanum*
267 neutralized only LBV. In our series, the highest rates with LBV across the different bat
268 species and islands were recorded on Mayotte and Mauritius (13.6% and 10.4% of the
269 samples, respectively) and in Malagasy *Myotis goudoti* and *Rousettus madagascariensis*
270 (36.4% and 20.0% of the samples, respectively).

271 Madagascar and islands making up the Comoros Archipelago are closer to the African
272 continent than the more outlying islands of the Mascarene and Seychelles archipelagos.
273 Among lyssavirus belonging to phylogroups I and II, only DUVV, SHIV, and LBV, have
274 been reported to date in African bats. Hence, one cannot exclude that DUVV and LBV are the
275 putative lyssaviruses actually circulating on SWIO islands. Interestingly, the reported
276 geographic distribution of DUVV, is primarily the northeastern part of South Africa,
277 Zimbabwe, Swaziland, and eastern Africa [24], i.e. in close proximity to the Mozambique
278 Channel and facing Madagascar. LBV has a much wider distribution, being isolated in
279 western, central, eastern, and southern Africa [27], not only from frugivorous bats (*Eidolon*
280 *helvum* and *Epomophorus gambianus*) but also from non-volant mammals, which develop
281 dead end infection. However, with the exception of a few species, the bat fauna of
282 Madagascar is largely endemic and holds a different suite of species than mainland Africa.

283 Alternatively, the putative bat lyssavirus(es) circulating on SWIO islands might be a
284 yet unidentified species. Over geological time, numerous bat groups colonized Madagascar by

285 over water dispersal from the African continent. The lack of subsequent genetic exchange
286 gave rise to divergence from these ancestral African lineages and the high levels of endemism
287 of Madagascar's bat fauna. This may have generated long-term co-evolution for a range of
288 hosts and their associated pathogens [37].

289 Our research shows for the first time, evidence of bat lyssavirus circulation on SWIO
290 islands, all previously deemed rabies free. In our samples, we did not detect by RT-PCR,
291 lyssavirus RNA in the brain of any insectivorous and frugivorous bat, whether the animal was
292 seropositive or seronegative. This result is not unanticipated, as no abnormal morbidity or
293 mortality among bats was observed during our study, and, in turn, the chance to isolate
294 viruses from healthy animals is reported to be very low [38]. The failure to detect any active
295 infection among animals, confirm that the seropositive individuals may have been infected
296 during an earlier stage of their life and able to quickly clear the virus once they developed
297 specific antibodies. The potential risk for human health with regard to contamination by bat
298 lyssaviruses is very low, though it should not be considered as nil. In the Old World, very few
299 cases of human rabies due to rabies-related bat lyssaviruses have been reported: only three
300 cases, all fatal, have been reported for DUVV [39] and not one associated with LBV.
301 However, the real number is unknown due to limited surveillance. Hence, an increased
302 awareness of the risk of rabies transmission through accidental or professional contact with
303 bats is recommended and any injury should be followed by a post exposure prophylaxy [40].

304

305 **Supporting Information**

306 **Figures S1-S6. Lyssaviruses neutralized by sera from bats captured on Anjouan (S1),**
307 **Mayotte (S2), Reunion Island (S3) Mauritius (S4), Mahé (S5), and Madagascar (S6).**
308 **(DOC)**

Text S1 Authorizations for sampling of bats on different western Indian Ocean islands (DOC)

Text S2. Ethical clearance (DOC)

Table S1. Sites names and corresponding abbreviations (DOC)

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339 **Contributions**

340 SMG, WM, and KD, conceived and designed the experiments. JM performed the
341 experiments. JM and KD analyzed the data. SMG, WM, SMC, BR, EL, and MT contributed
342 reagents/materials/analysis tools. JM, SMG, WM, SMC, HP, and KD and contributed to the
343 writing of the manuscript.

344 All authors reviewed the manuscript

345 **Competing interests**

346 The authors declare no conflict of interest.

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472 **Figure 1. Geographic distribution of bats collected on the SWIO islands of Anjouan**
473 **(Union of Comoros), Mayotte (France), Madagascar, Reunion Island (France),**
474 **Mauritius, and Mahé (Seychelles Archipelago).** n: number of bats sampled.

475

Figure S1-S6. Lyssaviruses neutralized by sera from bats captured on Anjouan (S1), Mayotte (S2), Reunion Island (S3) Mauritius (S4), Mahé (S5), and Madagascar (S6). Lyssaviruses neutralized by sera from bats captured on Anjouan (S1), Mayotte (S2), Reunion Island (S3) Mauritius (S4), Mahé (S5), and Madagascar (S6). Abbreviations in the pie diagrams correspond to each lyssaviruses (see Methods section). Abbreviations next to illustrated islands indicate names of capture sites (e.g. MGJ for "Mangajou") reported in Table S1A and Table S1B. In the pie diagram, n indicates at the numerator the number of sites with bats neutralizing or cross neutralizing (e.g. DUVV + EBLV-1) lyssaviruses and at the denominator the number of investigated sites; next to illustrated islands, n in the numerator indicates the number of individuals tested positive for lyssaviruses and in the denominator the number of individuals tested. For Madagascar, the six black squares indicate the provincial capitals.

488 **Table 1.** Seroreactivity of bats from southwestern Indian Ocean islands as assessed by RFFIT
489 using a panel of challenge lyssaviruses.

		Neutralization challenge viruses					Cross neutralization		
	Positive/tested	CVS-11	DUVV	EBLV-1	ABLV	LBV	CVS-11+ EBLV-1	CVS-11+ ABLV	DUVV+ EBLV-1
Anjouan	**4/21**	**0/8**	**2/8**	**0/0**	**0/0**	**2/21**	**0/0**	**0/0**	**0/0**
Molossidae									
Chaerephon pusillus	4/19	0/8	2/7	0/0	0/0	2/19	0/0	0/0	0/0
Miniopteridae									
Miniopterus griveaudi	0/2	0/0	0/1	0/0	0/0	0/2	0/0	0/0	0/0
Mayotte	**5/22**	**0/22**	**2/22**	**0/22**	**0/0**	**3/22**	**0/22**	**0/0**	**0/22**
Molossidae									
Chaerephon pusillus	2/3	0/3	1/3	0/3	0/0	1/3	0/3	0/0	0/3
Pteropodidae									
Pteropus seychellensis	3/19	0/19	1/19	0/19	0/0	2/19	0/19	0/0	0/19
Madagascar	**84/301**	**0/277**	**54/286**	**1/70**	**0/7**	**23/301**	**0/70**	**1/7**	**5/70**
Hipposideridae									
Hipposideros commersoni	3/6	0/6	1/6	1/4	0/0	0/6	0/4	0/0	1/4
Miniopteridae									
Miniopterus cf. *ambohitrensis*	4/17	0/15	4/15	0/4	0/0	0/17	0/4	0/0	0/4
Miniopterus gleni	0/4	0/4	0/4	0/0	0/0	0/4	0/0	0/0	0/0
Miniopterus griveaudi	12/33	0/23	10/29	0/6	0/0	2/33	0/6	0/0	0/6
Miniopterus mahafaliensis	0/11	0/9	0/11	0/0	0/0	0/11	0/0	0/0	0/0
Miniopterus sororculus	0/3	0/3	0/2	0/1	0/0	0/3	0/1	0/0	0/1
Molossidae									
Chaerephon atsinanana	0/16	0/16	0/15	0/0	0/0	0/16	0/0	0/0	0/0
Chaerephon leucogaster	10/28	0/26	9/28	0/6	0/0	0/28	0/6	0/0	1/6
Mops leucostigma	2/20	0/20	0/19	0/4	0/2	2/20	0/4	0/2	0/4
Mops midas	0/7	0/7	0/7	0/0	0/0	0/7	0/0	0/0	0/0
Mormopterus jugularis	20/58	0/58	17/58	0/8	0/0	3/58	0/8	0/0	0/8
Otomops madagascariensis	7/20	0/20	4/18	0/11	0/0	3/20	0/11	0/0	0/11
Pteropodidae									
Eidolon dupreanum	0/9	0/9	0/9	0/1	0/0	0/9	0/1	0/0	0/1
Pteropus rufus	8/12	0/10	5/11	0/5	0/0	0/12	0/5	0/0	3/5
Rousettus madagascariensis	12/35	0/33	4/33	0/16	0/5	7/35	0/16	1/5	0/16
Rhinonycteridae									
Triaenops menamena	2/11	0/7	0/11	0/2	0/0	2/11	0/2	0/0	0/2
Vespertilionidae									
Myotis goudoti	4/11	0/11	0/10	0/2	0/0	4/11	0/2	0/0	0/2
Reunion Island	**52/121**	**0/119**	**14/117**	**9/82**	**0/0**	**3/121**	**0/80**	**0/0**	**26/82**
Molossidae									
Mormopterus francoismoutoui	52/121	0/119	14/117	9/82	0/0	3/121	0/0	0/0	26/82
Mauritius	**28/67**	**0/62**	**19/67**	**2/29**	**0/5**	**7/67**	**0/29**	**0/5**	**0/29**
Molossidae									
Mormopterus acetabulosus	12/31	0/29	6/31	1/8	0/0	5/31	0/8	0/0	0/8
Pteropodidae									
Pteropus niger	16/36	0/33	13/36	1/21	0/5	2/36	0/21	0/5	0/21
Mahé	**13/40**	**0/40**	**6/40**	**1/17**	**0/2**	**4/40**	**1/17**	**0/2**	**1/17**
Pteropodidae									
Pteropus seychellensis	13/40	0/40	6/40	1/17	0/2	4/40	1/17	0/2	1/17
Grand Total	**186/572**	**0/528**	**97/540**	**13/220**	**0/14**	**42/572**	**1/218**	**1/14**	**32/220**

Figure
Click here to download Figure: renamed_1f90a.DOCX

Fig 1. Geographic distribution of bats collected on the SWIO islands of Anjouan (Union of Comoros), Mayotte (France), Madagascar, Reunion Island (France), Mauritius, and Mahé (Seychelles Archipelago). n: number of bats sampled.

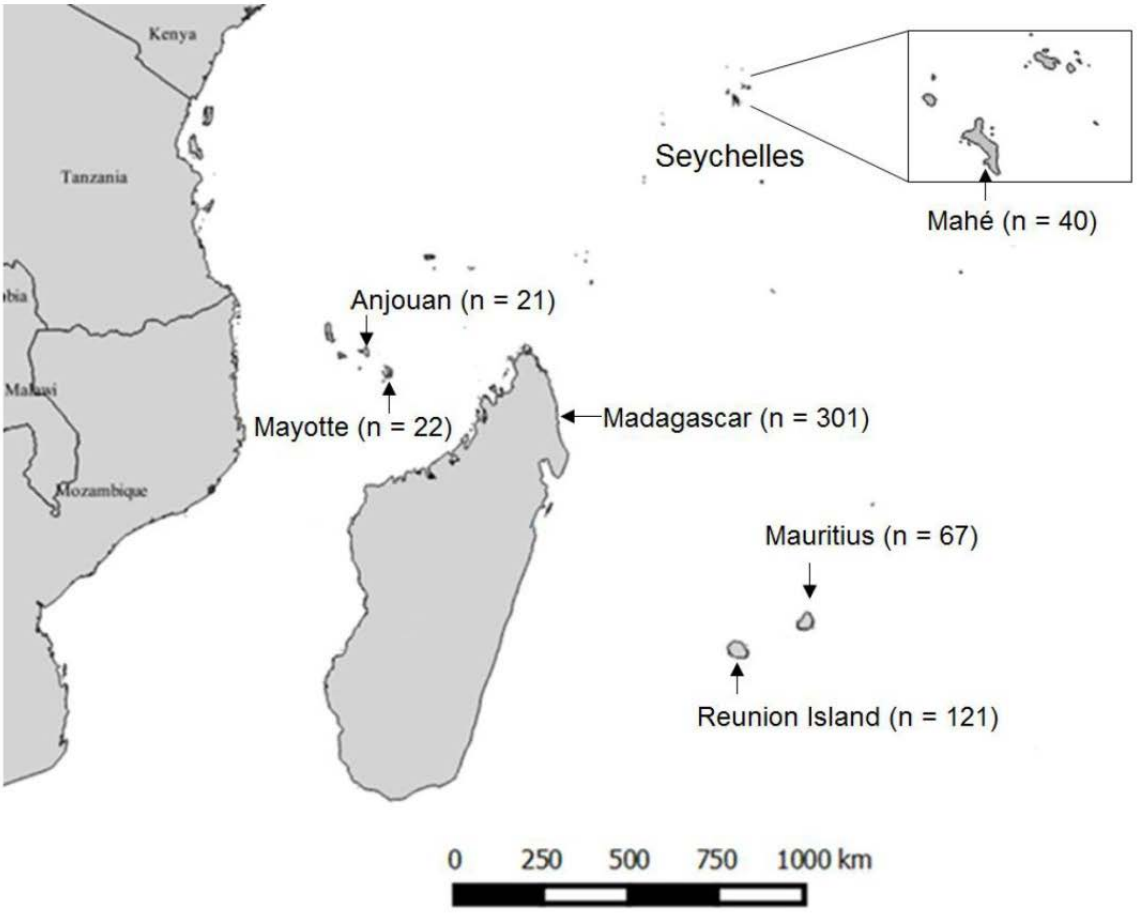

Figure S1-S6.

Fig S1.

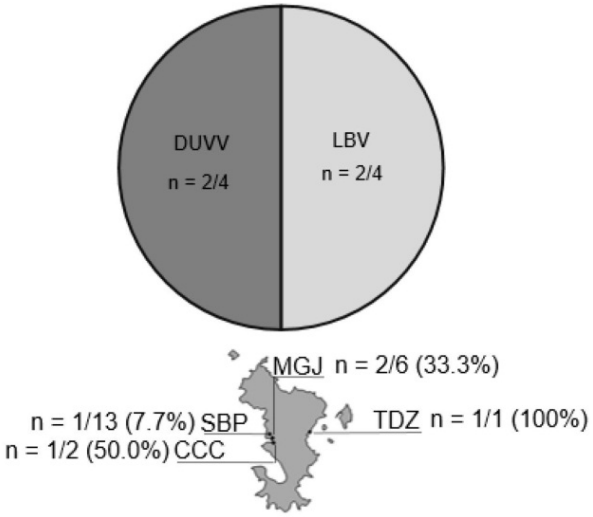

Fig S2.

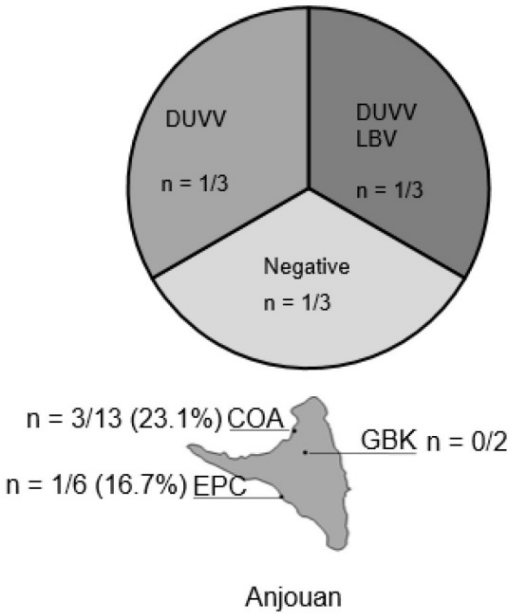

Fig S3.

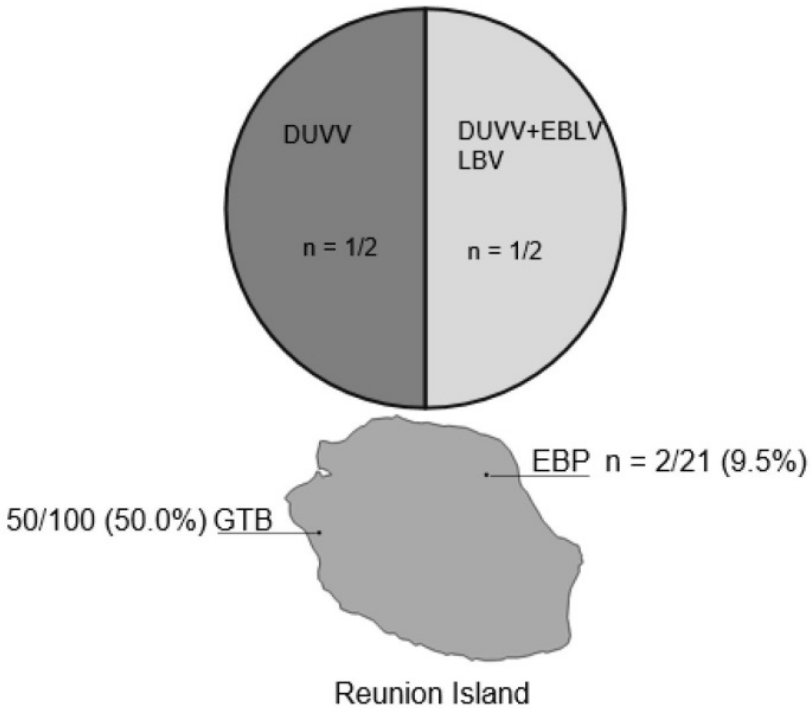
DUVV
n = 1/2
DUVV+EBLV
LBV
n = 1/2
EBP n = 2/21 (9.5%)
50/100 (50.0%) GTB
Reunion Island

Fig S4.

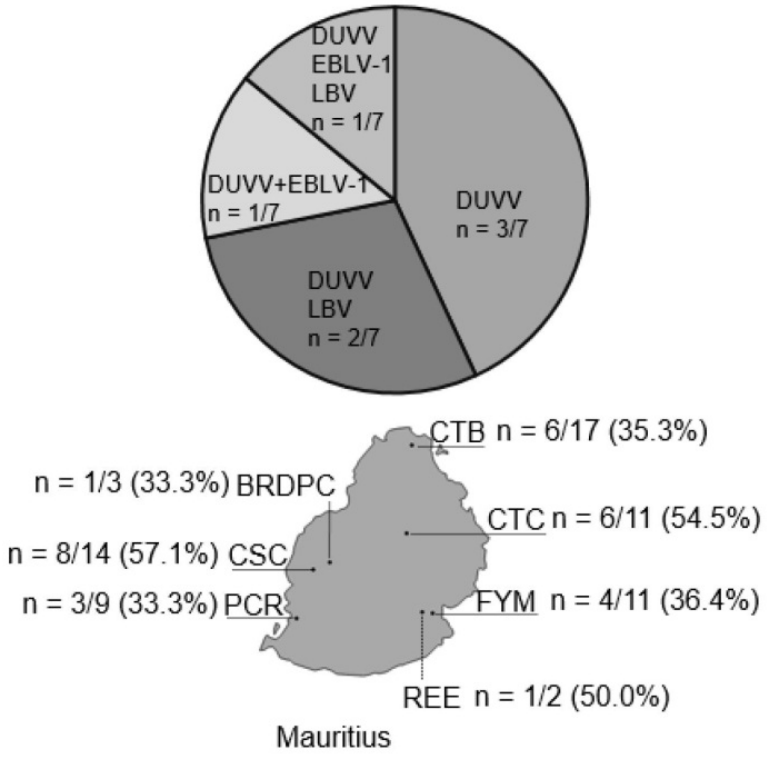
DUVV
EBLV-1
LBV
n = 1/7
DUVV+EBLV-1
n = 1/7
DUVV
n = 3/7
DUVV
LBV
n = 2/7
CTB n = 6/17 (35.3%)
n = 1/3 (33.3%) BRDPC
CTC n = 6/11 (54.5%)
n = 8/14 (57.1%) CSC
n = 3/9 (33.3%) PCR
FYM n = 4/11 (36.4%)
REE n = 1/2 (50.0%)
Mauritius

Fig S5.

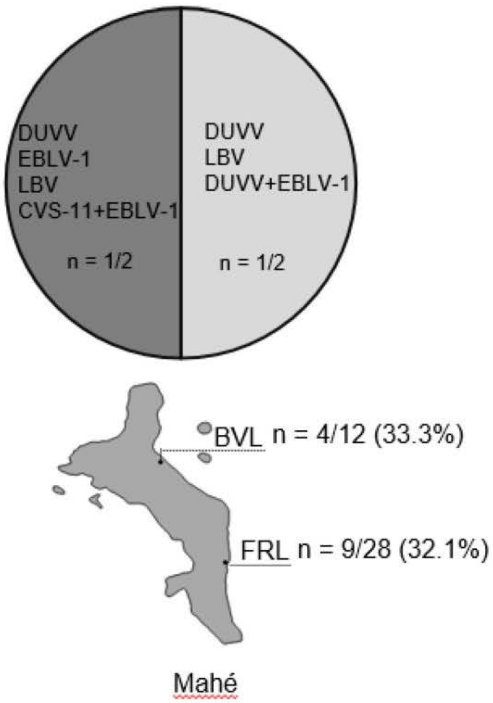
DUVV
EBLV-1
LBV
CVS-11+EBLV-1
n = 1/2
DUVV
LBV
DUVV+EBLV-1
n = 1/2
BVL n = 4/12 (33.3%)
FRL n = 9/28 (32.1%)
Mahé

Fig S6.

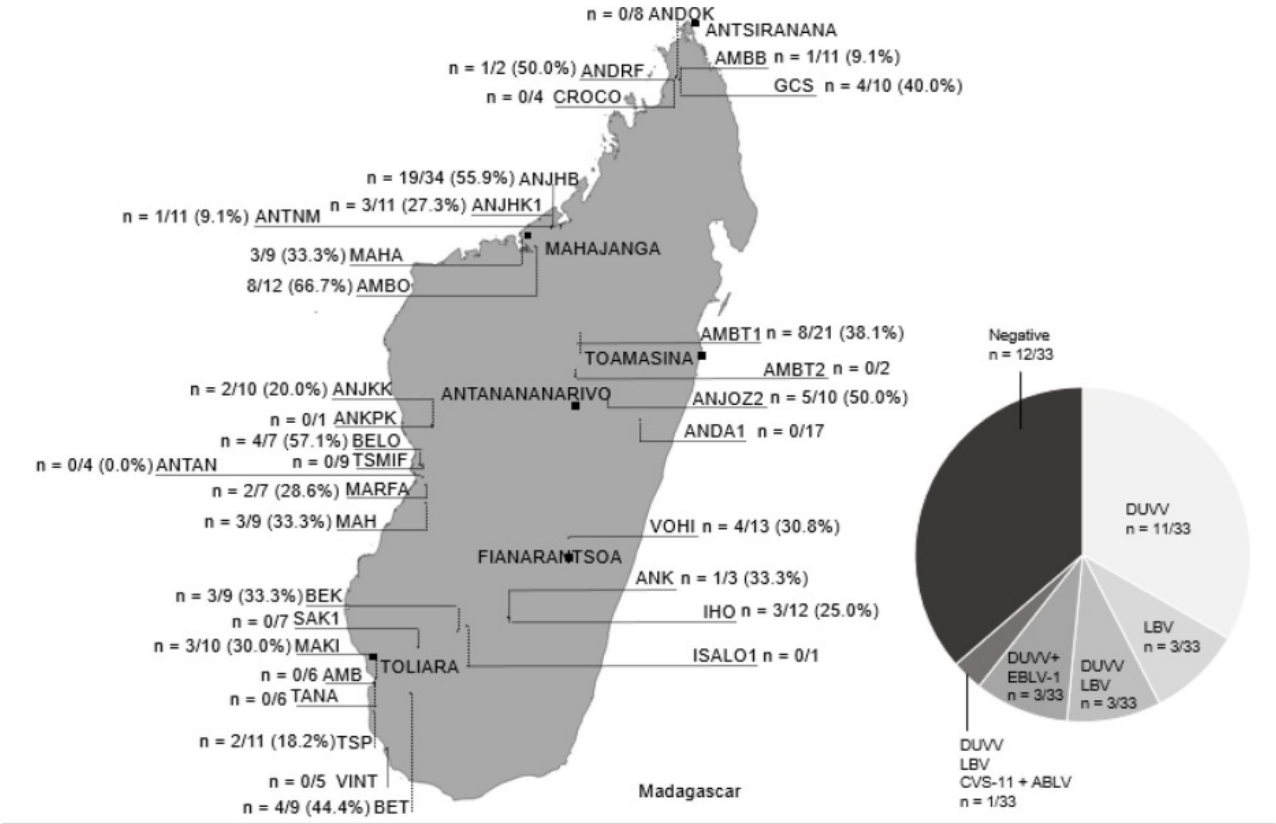
n = 0/8 ANDOK
ANTSIRANANA
AMBB n = 1/11 (9.1%)
GCS n = 4/10 (40.0%)
n = 1/2 (50.0%) ANDRF
n = 0/4 CROCO
n = 19/34 (55.9%) ANJHB
n = 3/11 (27.3%) ANJHK1
n = 1/11 (9.1%) ANTNM
MAHAJANGA
3/9 (33.3%) MAHA
8/12 (66.7%) AMBO
AMBT1 n = 8/21 (38.1%)
TOAMASINA
AMBT2 n = 0/2
n = 2/10 (20.0%) ANJKK
ANTANANANARIVO
ANJOZ2 n = 5/10 (50.0%)
n = 0/1 ANKPK
ANDA1 n = 0/17
n = 4/7 (57.1%) BELO
n = 0/4 (0.0%) ANTAN
n = 0/9 TSMIF
n = 2/7 (28.6%) MARFA
n = 3/9 (33.3%) MAH
VOHI n = 4/13 (30.8%)
FIANARANTSOA
ANK n = 1/3 (33.3%)
n = 3/9 (33.3%) BEK
IHO n = 3/12 (25.0%)
n = 0/7 SAK1
n = 3/10 (30.0%) MAKI
ISALO1 n = 0/1
TOLIARA
n = 0/6 AMB
n = 0/6 TANA
n = 2/11 (18.2%) TSP
n = 0/5 VINT
n = 4/9 (44.4%) BET
Madagascar
Negative
n = 12/33
DUVV
n = 11/33
LBV
n = 3/33
DUVV+
EBLV-1
n = 3/33
DUVV
LBV
n = 3/33
DUVV
LBV
CVS-11 + ABLV
n = 1/33

Table S1. Sites names and corresponding abbreviations

Abbreviations	Island	Site name
AMB	Madagascar	Grotte d'Ambanilia, 3.7 km SSE Sarodrano
AMBB	Madagascar	Parc National d'Ankarana, Ambahibe Cave, 2 km W Mahamasina
AMBO	Madagascar	Ambovondramanesy village near Berivotra, along RN4
AMBT	Madagascar	Réserve Spéciale d'Ambohitantely, Grotte des Chauves-souris
AMBT2	Madagascar	Réserve Spéciale d'Ambohitantely, début Sentier Touristique
ANDA1	Madagascar	CEG Andasibe
ANDOK	Madagascar	Parc National d'Ankarana, Grotte du troisiéme Canyon, along Andokotokana River
ANDRF	Madagascar	Parc National d'Ankarana, Grotte d'Andrafiabe, 3.3 ESE Andrafiabe
ANJHB	Madagascar	Grotte d'Anjohibe, 3.7 km NE Antanamarina
ANJHK1	Madagascar	Grotte d'Anjohikely (south entrance), 1.5 km NE Antanamarina
ANJKK	Madagascar	Parc National de Bemaraha, Anjohikinakina, 15.5 km N Bekopaka
ANJOZ2	Madagascar	Ambohibeloma, 3.2 km W. Anjozorobe
ANK	Madagascar	Commune rurale d'Ankily, west of Ihosy, off RN7
ANKPK	Madagascar	Ankapoka
ANTAN	Madagascar	Antanandava, Eglise FLM, 5.8 km NE Beroboka-Sud
ANTNM	Madagascar	Cascade d'Antanamarina
BEK	Madagascar	Parc National d'Isalo, Grotte de Bekapity
BELO	Madagascar	Belo Tsiribihina, central hospital
BET	Madagascar	Betioky-Sud, new Lutherian church
BRDPC	Mauritius	Black River District, Palma Cave
BVL	Mahé	Beau Vallon
CCC	Mayotte	Carrefour Chiconi
CGS	Madagascar	Parc National d'Ankarana, Grotte des Chauve-souris, 3 km NW Mahamasina
COA	Comoros	Anjouan, Collège d'Ouani
CROCO	Madagascar	Parc National d'Ankarana, 2.2 km ESE Amboandriky, Grotte d'Ambatoharanana (Crocodile Cave)
CSC	Mauritius	Black River District, Cascavelle

Table S1. Continued

Abbreviation	Island	Site name
CTB	Mauritius	Rivière du Rempart District, Caverne Trois Bras
CTC	Mauritius	Moka District, Camp Thorel, Camp Thorel Cave
EBP	Reunion Island	Eglise de Bras-Panon
EPC	Comoros	Anjouan, Ecole primaire de Chirove
FRL	Mahé	Fairy Land
FYM	Mauritius	Flacq District, northern slope Fayence Mountain
GBK	Comoros	Anjouan, Grotte de Buemokolo
GTB	Reunion Island	Grotte de Trois-Bassin
IHO	Madagascar	Ihosy, Bureau du chef de la Région,
ISALO1	Madagascar	Parc National d'Isalo, 3.8 km NW de Ranohira, along Namaza River
MAH	Madagascar	Mahabo, EPP de Mahabo
MAHA	Madagascar	North of Mahajanga, Petite Plage
MAKI	Madagascar	Grotte de Makis (Mikea), near Hotel la Mangrove, on Toliara-St Augustin Road
MARFA	Madagascar	Marofandilia, Ecole primaire
MGJ	Mayotte	Mangajou
PCR	Mauritius	Black River District, Petite Case Noyale
REE	Mauritius	Grand Port District, Riche en Eau
SAK1	Madagascar	Sakaraha, Direction des Eaux et forêt, Bureau chef de cantonnement
SBP	Mayotte	Sohoa be plage
TANA	Madagascar	Grotte de Tanambao (Bishiko), 0.75 km E St Augustin
TDZ	Mayotte	Tsoundzou
TSMIF	Madagascar	Tsimafana, CEG de Tsimafana
TSP	Madagascar	Parc National de Tsimanampetsotsa, Grotte d'Andranoilovy
VINT	Madagascar	Grotte de Vintane (Vintany), 4.1 km SE Itampolo
VOHI	Madagascar	Vohipoa, CSB II

Text S1. Authorizations for sampling of bats on different western Indian Ocean islands

1. Madagascar: Direction du Système des Aires Protégées and Direction Générale de l'Environnement et des Forêts; Madagascar National Parks: Export permit n°194/12/MEF/SG/DGF/DCB.SAP/SCB, 067/12/MEF/SG/DGF/DCB.SAP/SCBSE, and 032/12/MEF/SG/DGF/DCB.SAP/SCBSE. A CITES permit from the Malagasy national authority was issued for tissue export (permit 243C-EA06/MG12) to CRVOI on Reunion Island.
2. Reunion Island: Préfecture de La Réunion: Arrêté préfectoral of 11 Février 2013.
3. Mayotte: Préfecture de Mayotte: Arrêté préfectoral n°158/DEAL/SEPR/2014.
4. Anjouan, Union of Comoros: Centre National de Documentation et de Recherche Scientifique (CNDRS) of Union des Comores: Export permit CNDRS 021/10.
5. Mahé, Seychelles Archipelago: Direction of Wildlife, Trade and Conservation Section and the Ministry of Environment and Energy of Republic of Seychelles: Export permits Agreement of 5 March 2014. CITES permit from the Republic of Seychelles was issued for export (permit N°1772) to CRVOI on Reunion Island.
6. Mauritius: National Park and Conservation Service for authorization of Mauritius: Memorandum of agreement for the supply of biological material by Government of Mauritius, signed 17 December 2010 and 09 January 2013. CITES permit from the Mauritian national authority was issued for tissue export (permit MU120933) to CRVOI laboratory on Reunion Island.

6.4. Conclusion du chapitre 6

Dans cette étude nous avons mis en évidence une circulation sérologique importante des lyssavirus chez les chauves-souris de toutes les îles de la zone du SOOI. Un très grand nombre d'espèces de chauves-souris (plus de 12 espèces insectivores et 2 espèces de frugivores) a été retrouvé séropositif suggérant un large spectre d'hôte pour ces lyssavirus. Les taux d'infection les plus élevés ont été observés à La Réunion, Maurice et Mahé, et des taux les plus faibles à Madagascar, Mayotte et à Anjouan. Les profils de séroneutralisation sont en faveur de la circulation d'au moins deux lyssavirus, l'un appartenant au phylogroupe I (rendant compte des réactivités croisées DUVV, EBLV et ABLV), et l'autre, au phylogroupe II (rendant compte de la réactivité croisée avec LBV).

Cependant, aucune trace d'ARN n'a été mise en évidence chez les chauves-souris séropositives ou séronégatives comme l'indique les résultats de l'analyse moléculaire qRT-PCR sur les cerveaux d'animaux. Cela suggère qu'une exposition antérieur des chauves-souris vis-à-vis d'un lyssavirus du phylogroupe I et / ou du phylogroupe II a eu lieu mais que celui-ci pourrait avoir été éliminé par le système immunitaire ou serait présent mais à très faible concentration. Des travaux complémentaires sont nécessaires, en particulier sur les chauves-souris malades ou morbides ainsi que sur les glandes salivaires pour pouvoir identifier les lyssavirus qui circulent dans la région.

Ces travaux ont été valorisés sous forme d'un article scientifique soumis et de trois présentations orales au congrès international « **African Small Mammal Symposium** » (ASMS, 2015), et aux Journées scientifiques de la « **Fédération Environnement, Biodiversité** » et Santé (FedEBS ; 2014, Saint-Denis La Réunion), ainsi qu'au Colloque **FEDER POCT Biodiversité** (2015, Saint-Denis La Réunion).

Annexe 6 : Les résultats obtenus sur la sérologie Panvirale nous ont permis de détecter de fortes séroprévalence sen *Paramyxoviridae*, *Bunyaviridae* et *Flaviviridae* chez les chauves-souris, petits mammifères terrestres endémiques et rongeurs. Les séroprévalences en *Cornoraviridae* et *Togaviridae* étaient les plus faibles. Les individus fortement séropositifs appartiennent à la famille des Pteropodidae (chauves-souris frugivores du genre *Eidolon*, *Pteropus* et *Rousettus*).

Enfin, les analyses montrent une plus forte séroprévalence contre les *Rubulavirus*, notamment *Mumps* et *PIV-2*, au sein des individus collectés en particulier chez les chauves-souris frugivores. Cela suggère une circulation active d'un virus putatif proche de ce genre viral en plus des *UMRVs* au sein des colonies de frugivores.

L'ensemble de ces résultats témoigne d'une forte sensibilité des chauves-souris de la zone du SOOI aux virus à ARN potentiellement zoonotiques, et particulièrement, les virus des familles *Paramyxoviridae*, *Flaviviridae* et *Bunyaviridae*.

Ces travaux seront valorisés sous forme d'un article scientifique qui est en cours de soumission.

Discussion générale

Les déterminants écologiques et les processus évolutifs qui entrent en jeu dans les phénomènes de "*host-switch*" des agents infectieux zoonotiques issus de la faune sauvage vers les populations humaines et d'animaux domestiques ou de compagnie sont encore mal connus. Etant donné que 80% des virus émergeants qui concernent l'homme proviennent de la faune sauvage (Taylor et al., 2001), une attention croissante leur sont accordés au niveau mondial par les scientifiques et les autorités sanitaires, en raison des enjeux majeurs socio-économiques et de leurs impacts sur les politiques publiques de santés humaine et vétérinaire. Les graves épidémies toujours en cours provoquées par le virus *Ebola* en Afrique de l'Ouest (CDC, 2015) ou du MERS-CoV au Moyen Orient (CDC, 2014), ainsi que les flambées épidémiques de la PPR (Ishag et al., 2015) qui dévastent les cheptels des petits ruminants d'Asie et d'Afrique attestent bien de l'urgence de la situation (OMS, OIE, FAO). Anticiper la survenue de telles émergences infectieuses et comprendre les tenants et les aboutissants des facteurs qui y concourent passe par l'exploration systématique des réservoirs sauvages impliquant à la fois l'étude de leur l'écologie et des mécanismes évolutifs des agents infectieux qui leurs sont associés (Morse et al., 2012).

Mes travaux de thèse s'inscrivent dans le cadre de l'approche éco-systémique conduite par le CRVOI sur les maladies infectieuses émergentes et font suite aux premières informations obtenues lors de l'exploration de certaines îles du SOOI (Madagascar, Mayotte et La Réunion) sur certains réservoirs animaux potentiels tels que les chauves-souris (Wilkinson et al., 2012). Ces travaux avaient mis en évidence une diversité importante de nouveaux paramyxovirus, avec des prévalences élevées en comparaison à d'autres études (Kurth et al., 2012 ; Sasaki et al., 2012).

Face à ces observations saisissantes, mes travaux de thèse ont abordé la question de la dynamique de transmission virale et ont tenté de mieux caractériser ces virus potentiellement zoonotiques circulant parmi les micromammifères terrestres et volants de la zone du SOOI. Les informations préliminaires laissaient penser que de multiples échanges viraux inter-îles, surviennent entre des hôtes très divers dans la région du SOOI.

Cette région multi-insulaire est singulière de par sa grande biodiversité et un fort endémisme en lien avec des particularités écologiques (Tortosa et al., 2012).

Nous avons ainsi pu mettre en évidence plusieurs résultats originaux : (i) une circulation importante de nouveaux paramyxovirus (*UMRVs*), appartenant probablement à un nouveau genre viral phylogénétiquement très apparenté aux *Morbillivirus,* ayant une aptitude à infecter un large spectre d'hôtes, appuyant ainsi le rôle de réservoir de ces mammifère, incluant Chiroptera, Rodentia et Afrosoricidae ; (ii) la co-divergence génétique des *UMRVs* ne peut pas être le résultat d'un mécanisme de co-spéciation des hôtes, mais résulte principalement du mécanisme du *host-Switch* en terme macroévolutif chez les chauves-souris ; (iii) des facteurs écologiques jouent un rôle important dans la dynamique de transmission virale ; (iv) enfin de façon plus générale, l'analyse sérologique révèle une intense circulation régionale de lyssavirus de chauves-souris dans un très grand nombre d'espèces, virus qui se rattachent très probablement pour l'un au phylogroupe I et pour l'autre au phylogroupe II. Il n'est d'ailleurs pas impossible, considérant l'isolement relatif des îles du SOOI, que l'un ou l'autre se révèlent *in fine* de nouveaux membres de ce genre viral et qu'ils soient spécifiques des espèces endémiques de chiroptères retrouvées uniquement dans le SOOI.

Les processus évolutifs mis en jeu

Macro-évolution

Une caractéristique remarquable commune aux deux modèles viraux, paramyxovirus et lyssavirus, est le large spectre d'hôtes. En effet, plus de 15 espèces de chauves-souris (6 familles) et au moins 10 espèces de petits mammifères terrestres endémiques (2 familles) sont infectées par les *UMRVs* et 14 espèces de chauves-souris (6 familles) sont concernées par un putatif lyssavirus. Ces résultats confirment le rôle des chauves-souris et petits mammifères terrestres en tant que réservoir viral majeur (Calisher et al., 2006 ; Meerburg et al., 2009 ; Drexler et al., 2012 ; Banyard et al., 2014). A ce stade de nos investigations, certaines questions méritent d'être posées : Quelles sont les raisons qui permettent à des virus aussi génétiquement divers, d'infecter des hôtes si diversifiés ? Existe-t-il à l'origine d'un tel spectre, un mécanisme évolutif global au niveau viral, ou est-ce la conséquence de facteurs écologiques ? Le moteur macro-évolutif (à savoir le mécanisme de *host-switch*) que nous avons démontré chez les paramyxovirus de chauves-souris répond en grande partie à cette question.

Ce même mécanisme de co-divergence virale associée à des évènements de sauts d'espèces a été rapporté chez d'autres agents zoonotiques viraux issus de la faune sauvage (Ramsden et al., 2009 ; Coulibaly-N'Golo et al., 2011), mais aussi pour les Paramyxovirus infectant l'homme (Kitchen et al., 2011).

Au cours de nos travaux, nous avons tiré profit de la diversité d'espèces de chauves-souris présentes à Madagascar afin de déterminer si la diversité des paramyxovirus était issue de celle des hôtes (spéciation d'hôte entraînant une spéciation du virus) ou serait propre aux paramyxovirus eux-mêmes. Ainsi, en comparant 7 familles et 31 espèces de chauves-souris Malgaches différentes, nous avons montré que les multiples phénomènes de sauts d'espèces prennent le pas sur l'hypothèse d'une macro-évolution directement liée à une co-spéciation de l'hôte. Ce dernier mécanisme a longtemps (et le reste encore pour beaucoup d'auteurs) été avancé comme le principal mécanisme macro-évolutif en œuvre chez de nombreux modèles viraux (de Vienne et al., 2013). Il est important de mentionner que la plupart des algorithmes de co-phylogénie ont été mis au point sur des modèles bactériens et *de facto* ne sont pas ou mal adaptés, à l'analyse des modalités évolutives chez les virus ARN. Ces logiciels s'appuient sur des algorithmes essentiellement basés sur des processus de co-spéciation (Merkle & Middendorf, 2005), et nous pensons que c'est l'une des raisons pour lesquels ils aboutissent à une surreprésentation de ces derniers: derrière une apparente congruence phylogénétique (phénomènes d'associations hôtes-virus), entre des agents infectieux et leurs hôtes, il ne pouvait s'agir que d'une co-divergence virale liée à la co-spéciation de l'hôte (Merkle et al., 2010). En comparaison, si on se réfère à des modèles bactériens, comme chez les Leptospires, ou encore sur des modèles parasitaires plus complexes, tels que les Hémoparasites (plasmodium et filaire), modèles développés par mes collègues au sein du CRVOI à partir du même échantillonnage d'hôtes (chauves-souris) (respectivement thèse de Yann Gomard et post-doctorat de Beza Ramasindrazana), les très fortes associations entre les bactéries/hémoparasites et leurs hôtes respectifs dans leurs cas, révèlent que les relations évolutives hôtes/leptospires/hémoparasites sont dominées par le mécanisme de co-spéciation des hôtes, entrainant à chaque étape de spéciation, la co-divergence des agents infectieux correspondants. Quelque part, une telle discordance entre ces modèles était attendue s'agissant de bactéries et de parasites qui n'élaborent pas les mêmes stratégies du point de vue évolutif. Ces agents infectieux diffèrent en termes de structure, de cycles de vie, de mode de réplication/multiplication, et surtout, au niveau de leur horloge moléculaire respective.

Les bactéries et les parasites ont des taux d'évolution très lents (de l'ordre de 10^{-9} à 10^{-6} substitutions/site/an) comparés à ceux des virus à ARN (de l'ordre de 10^{-5} - 10^{-3} substitutions/site/an) (Holland et al., 1982), Ces derniers, accumulent des mutations de manière beaucoup plus importante en raison de leur taux de réplication élevé et du fait de l'absence de l'activité correctrice 3'-5' de leur ARN polymérase ARN-dépendante (Duffy et al., 2008) produisant ainsi des populations virales hétérogènes complexes (quasi-espèces) (Holmes & Moya, 2002 ; Domingo et al., 2005).

Microévolution

D'après certains auteurs, la variabilité génétique qui caractérise les virus à ARN, serait intimement liée aux mécanismes micro-évolutifs, c'est-à-dire aux relations d'adaptations complexes qui s'établissent lors des interactions hôtes-pathogènes au sein de communautés d'espèces, par exemple (Page, 2003 ; Jackson et al., 2004 ; Woolhouse et al., 2001). Elle pourrait expliquer la diversité virale observée au sein des *UMRVs*. Une étude récente a par ailleurs incriminé la plasticité des génomes des virus à ARN comme pouvant être à l'origine du « *host-switch* » (Kreuder Johnson et al., 2015). Comme décrit dans le chapitre I, après chaque « *host-switch* » viral, il est nécessaire pour le virus de s'adapter à son nouvel hôte, au risque de connaitre l'extinction. Les façons de s'adapter à son nouvel environnement sont diverses tels que celui de varier (variant de mutation) ou encore de procéder à des recombinaisons, permettant ainsi d'obtenir des variants qui seraient plus à même de reconnaitre et de se lier à des récepteurs "alternatifs" permettant des infections de nouveaux types cellulaires, ou encore modifier la pathogénicité (Baranowski et al., 2003). Les dispositifs ainsi mis en jeu peuvent permettre au virus d'échapper ou de contourner les défenses immunitaires de l'hôte nouvellement infecté (échappement viral) (Ciurea et al., 2000). Quelles sont ces mutations et à quelles fréquences apparaissent-elles ? Sont-elles toutes les mêmes pour tous les virus ? Sur quelle partie précise du génome se produisent-elles ? Comment les paramyxovirus infectent ils et se reproduisent ils dans de multiples hôtes ? Ya t'il en définitive un phénomène de persistance virale ? En effet n'ayant pu conduire d'étude longitudinale sur une cohorte de chauves-souris ou de rongeurs sauvages, nous ne pouvons formellement répondre à cette question.

Les récepteurs étant des éléments essentiels dans les premières étapes d'infection et, les paramyxovirus infectant un très grand nombre d'espèces différentes, ils devraient reconnaitre un récepteur "ubiquitaire" chez les espèces communes concernées qui reste à déterminer pour ce qui concerne les *UMRVs*. Il convient de signaler que la conservation des récepteurs et voies cellulaires entre chauves-souris et différents mammifères est suspectée comme l'élément moteur de la transgression de la barrière d'espèce facilitant la transmission de virus à d'autres taxons (Calisher et al., 2006).

Comme décrit dans la littérature (Anishchenko et al., 2006 ; Orton et al., 2013), il serait très intéressant de caractériser ces mutations et ces récepteurs cellulaires ubiquitaires qui vont permettre la persistance et l'adaptation virale. Le séquençage complet des génomes des *UMRVs* qui est en cours d'analyse devrait en partie nous aider à répondre à ces interrogations. Quoiqu'il en soit, de tels mécanismes pourraient probablement expliquer la grande diversité virale des *UMRVs*, laquelle résulterait, d'une part, de leur longue histoire évolutive, jalonnée au niveau macro-évolutif, par de multiples sauts d'espèces, et sans cesse compensée, au niveau micro-évolutif, par des mécanismes d'adaptation au sein des nouveaux hôtes.

On sait que les hôtes phylogénétiquement proches entres eux sont plus à risque de *host-switch*, notamment, du fait de la forte conservation entre leurs récepteurs cellulaires de surface (Longdon et al., 2011). C'est le cas pour le virus rabique et pour les hantavirus, où des études montrent que le *host-switch* viral se produit préférentiellement entre des espèces de chauves-souris et rongeurs phylogénétiquement très proches (Ramsden et al., 2009 ; Steicker et al., 2010). Nos résultats (Wilkinson et al., 2014) démontrent qu'il est aussi possible que des paramyxovirus phylogénétiquement très proches, peuvent infecter des hôtes phylogénétiquement éloignés: dynamique infectieuse et échanges viraux inter-ordres, Chiroptera et Rodentia. Un tel phénomène a déjà était décrit par l'équipe de Guo et al. (2014) qui démontré que le phénomène de « *host-switch* » s'est produit depuis la chauves-souris, décrit comme le réservoir principal des hantavirus dans son étude, vers les rongeurs.

Le rat noir (*Rattus rattus*) et le rat brun (*Rattus norvegicus*), originaires d'Inde et d'Asie rspectivement, se sont ensuite rapidement dispersés dans le monde (Aplin et al., 2003). Ces espèces sont réservoirs de très nombreux virus et surtout décrits comme disséminateurs de parasites et de virus (Wilkinson et al., 2014 ; Firth et al., 2014 ; Morand et al., 2015).

On sait notamment qu'à Madagascar, les rongeurs introduits sont présents à proximité des entrées de grottes de chauves-souris. Il est envisageable qu'une transmission virale chauves-souris / rongeurs ait lieu et que celle-ci passe par l'ingestion d'eau ou d'aliments contaminés par l'urine ou les fèces de chauves-souris par exemple. Nos analyses ont également montré un échange de paramyxovirus entre l'Afrique et le SOOI (Wilkinson et al., 2014). Comme décrit dans le chapitre I, *Rattus rattus* est connu pour être une espèce invasive largement distribuée à travers le monde (Lund et al., 1994 ; Aplin et al., 2003). Les transports maritimes ont contribué en grande partie à sa dissémination mondiale, et entre pays très éloignés (Bonnefoy et al., 2008). De fait, les échanges commerciaux des îles du SOOI se produisent avec l'Asie, l'Europe ou l'Afrique. Par ailleurs, l'étude conduite sur les paramyxovirus des rongeurs indigènes à la Tunisie, en Afrique du Nord (*Psammomys obesus, Meriones shawi* et *Ctenodactilus gundii*) a montré leur proximité phylogénétique avec des paramyxovirus des Iles du SOOI (Ghawar et al., soumis). De plus nous avons pu retracer l'histoire évolutive de ces paramyxovirus, montrant qu'ils seraient originaires d'Afrique australe, et qu'ils ont été disséminés très vraisemblablement par les rongeurs introduits *Rattus*. Ainsi *R. rattus* aurait joué le rôle de pont épidémiologique entre les différents hôtes, mais également entre toutes les régions du globe.

Par ailleurs, en plus des *host-switch* fréquemment observés, nous avons mis en évidence quelques cas d'associations entre hôtes et *UMRVs*. Du fait que les virus évoluent rapidement, le système immunitaire de l'hôte doit lui aussi s'adapter, notamment par l'évolution rapide simultanée des gènes de ses récepteurs à l'antigène lui permettant de moduler continuellement sa réponse face à ses « cibles changeantes ». Ces situations conflictuelles créent une course sans fin aux armements entre l'hôte et le virus (van Valen, 1973). Il s'agit en partie de l'hypothèse évolutive de la "Reine Rouge" dans laquelle l'évolution des gènes de l'immunité doit toujours rester au même niveau que celle des virus. L'alternative adoptée par certains virus, serait que, sous certaines pressions de sélection du système immunitaire de l'hôte, certains virus vont être "séquestrés" au niveau de différents compartiments (niches) à l'intérieur d'un hôte (Kitchen et al., 2011) et s'y spécialiser, générant une association entre l'hôte et le virus extrêmement forte et étroite. Ce type d'adaptation peut conduire le pathogène vers une "hyperspécialisation" ou une sanctuarisation dans un tissu ou un type cellulaire donné. Ce phénomène pourrait représenter une barrière évolutive moins "permissive" que celle qui ferait suite à l'infection par *host-switch* d'un pathogène pour un nouvel hôte.

Facteurs éco-épidémiologique

De nombreuses études ont montré la présence de paramyxovirus appartenant au genre *Henipavirus* chez les chauves-souris frugivores, principalement du genre *Pteropus* ou *Eidolon* (Field et al., 2012 ; Drexler et al., 2009 ; Yadav et al., 2015 ; Chua et al., 2000). Nos résultats sont différents de ces études puisque nous décrivons une très grande proportion de chauves-souris insectivores infectées, et une très faible proportion de chauve-souris frugivores infectées, et pour celles qui le sont, l'infection est toujours due par un virus appartenant au genre des *UMRVs*. Cette restriction à ce genre unique doit cependant être nuancée dans la mesure ou les autres genres de paramyxovirus (*Pneumovirus*, *Avulavirus* et *Rubulavirus*) ne sont pas détectables par le système de PCR nichée que nous avons utilisée. En tout état de cause, nous ne pouvons que souligner l'absence de détection de virus Henipa dans la région du SOOI.

L'analyse multivariée a mis en évidence une transmission virale plus active au sein des chauves-souris insectivores dans notre étude, puisque sur 87 chauves-souris frugivores, seulement 3 d'entre elles, du genre *Pteropus*, ont été retrouvées positives, démontrant que les *UMRVs* infectent préférentiellement le groupe des insectivores. Les Yinpterochiroptera (chauves-souris insectivores) représentent la sous famille la plus diversifiée à Madagascar et la plupart des individus phylogénétiquement proches, et plus important encore, la plupart des genres Miniopteridae sont retrouvés en sympatrie sur le même site.

Notre analyse multivariée a en effet montré que le comportement grégaire des chauves-souris, et principalement la sympatrie, sont des facteurs clés dans les prévalences fortes en paramyxovirus chez les chauves-souris. Ce patron a été observé à la fois pour des chauves-souris de la même famille ou de familles différentes. Un tel phénomène a déjà été soulevé par l'équipe de Luis et al. (2014) qui démontre que le contact étroit entre différentes espèces de chauves-souris sur un même site, trait écologique caractéristique des chiroptères, favoriserait la transmission virale entre différentes espèces. Dans une étude ultérieure, ces mêmes auteurs ont montré que le comportement grégaire était lui aussi corrélé à la richesse virale au sein des chauves-souris. Par ces résultats nous démontrons l'importance de la biodiversité, à savoir une richesse d'hôtes au sein d'une communauté de chauves-souris, les Yinpterochiroptera, accompagnés des traits de vie écologiques, la sympatrie, en tant que facteur amplificateur de la transmission virale.

Keesing et al ont décrit l'effet favorable ou défavorable que pouvait avoir la biodiversité sur la transmission d'agents pathogènes (Keesing et al., 2010). Dans notre modèle d'étude, nous montrons que la diversité d'espèces est corrélée à la diversité virale, ce qui d'après Morse et al (Morse et al., 1993) favoriserait la génération de "pools zoonotiques". Un dernier facteur écologique important relevé dans notre étude est l'effet de la température plus élevée dans certaines localités de Madagascar qui apparait comme facteur de risque dans la transmission de paramyxovirus chez les chauves-souris de Madagascar. Des études intéressantes avaient déjà révélé l'importance du climat sur le taux d'évolution du virus de la rage (Streicker et al., 2012). Ainsi le virus rabique retrouvé chez des chauves-souris des régions tropicales et subtropicales aurait un taux d'évolution quatre fois plus important que chez les chauves-souris vivant en zone tempérée. Il est apparu que chez les chauves-souris vivant dans les régions plus chaudes, le métabolisme énergétique serait accéléré, augmentant potentiellement le taux de mutations dû à la production massive de radicaux libres (Rohde et al., 1992 ; Martin & Palumbi, 1993). A l'inverse, les chauves-souris qui entreraient en hibernation/torpeur dans les régions froides présentent des taux d'infections très bas sans doute dû à une diminution du métabolisme cellulaire (Domingo et al., 1997). Au vu du nombre de « *host-switch »* observé inter- et intra-ordres de micromammifères dans la zone SOOI, il est fort probable qu'en couplant nos analyses écologiques aux analyses génétiques, nos résultats pourraient indiquer un effet positif de la température élevée sur le taux d'évolution viral expliquant *in fine*, la quantité importante de *host-switch* observée dans la région SOOI.

Enfin, une étude réalisée par l'équipe du CRVOI en 2014 a décrit la saisonnalité, notamment, des périodes de reproduction des chauves-souris, comme étant un facteur important dans la dynamique de transmission d'agent pathogène à La Réunion (Dietrich et al., 2014) en accord avec d'autres études (O'Shea et al., 2014). L'étude menée à La Réunion sur l'espèce endémique *Mormopterus francoismoutoui* retrouvée dans une grotte dédiée à la maternité à La Réunion a montré une dynamique infectieuse saisonnière où la transmission des paramyxovirus semble intervenir de façon active en deux pics; le premier pic d'infection est observé à la fin de la grossesse et concerne préférentiellement les mères gestantes au moment où elles se concentrent à très forte densité dans la grotte maternité et sont le plus en contact entre elles. Le taux d'infection des chauves-souris peut atteindre 50%.

Un second pic d'infection survient deux mois après l'accouchement, et concerne les juvéniles quelques semaines après leur naissance, vraisemblablement après la perte des anticorps maternels passivement transmis lors de la période d'allaitement (Dietrich et al., 2014). Les séquences génétiques correspondant aux *UMRVs* excrétés dans les urines ont été trouvées similaires des séquences des *UMRVs* retrouvés dans le surnageant de broyat de pool d'organes (reins, poumon et rate) (données non publiés). Il est envisageable que l'excrétion des paramyxovirus chez les chauves-souris coïncide avec une infection systémique, i.e. avec une virémie transitoire. Cependant une telle possibilité n'est pas exclusive d'une excrétion virale plus prolongée dans le temps et non corrélée avec la virémie, une fois que l'infection rénale chronique se serait établie chez les animaux qui deviennent de ce fait excréteurs de virus, i.e. un hôte réservoir. Par ailleurs, les résultats de cette étude suggèrent que la dynamique virale pourrait être modulée par la réponse immune développée par l'animal infecté. Ces résultats très intéressants permettent d'orienter les futures investigations par biologie moléculaire sur des prélèvements récoltés de manière non invasive telle que l'urine afin d'identifier la fraction des chauves-souris qui jouerait le rôle d'hôtes de maintien du virus et permettant une excrétion virale sur de longues périodes, du moins d'une saison sur l'autre entre deux épisodes d'accouchement, pour des raisons (immunologiques ?) qui restent encore à clarifier.

Système immunitaire

Un aspect intéressant encore peu étudié, est le système immunitaire des chauves-souris. Les chauves-souris sont des mammifères très anciens, près de 80 Millions d'années. Il est possible que leurs réponses immunitaires innées et acquises soit différentes des autres mammifères mieux étudiés (rats, souris). Comme décrit dans le chapitre I, on sait que des virus extrêmement pathogènes comme *Ebola* et *SARS* qui provoquent respectivement fièvres hémorragiques et pneumonies, sont retrouvés chez des chauves-souris saines (Biek et al., 2006 ; Wang et al., 2006). Les réponses immunitaires de celles-ci sont-elles régulées différemment permettant de contrôler le niveau de réplication du virus, sans l'éliminer totalement ? (Calisher et al., 2006). Pourquoi certains virus infectent-ils et persistent-ils chez les chauves-souris alors qu'ils se révèlent hautement pathogènes pour les humains et les autres vertébrés? Pourquoi certaines espèces de chauves-souris infectées expriment elles des symptômes et d'autres pas ?

Quel est le rôle du système immunitaire dans le maintien de l'infection virale? Malheureusement très peu de données sont disponibles sur ces points cruciaux.

L'hypothèse d'un mutualisme a été émise dans lequel le système immunitaire imposerait un équilibre qui maintiendrait l'infection et la transmission d'un virus à un niveau basal favorisant à la fois l'hôte et l'agent pathogène (Calisher et al., 2006). Ce trade off expliquerait aussi l'absence de symptômes chez les individus collectés dans cette étude puisque nous n'avons jamais observé d'individu apparemment malade ni de mortalité anormale sur les sites visités. Les lyssavirus qui sont les virus le plus souvent impliqués dans la transgression de la barrière d'espèces, sont capables d'infecter un spectre d'hôte extrêmement élevé (Banyard et al., 2014). L'absence de détection d'ARN ou d'antigène viraux, malgré la très forte séropositivité est une observation courante rapportée dans la plupart des études portant sur les lyssavirus (Arguin et al., 2002 ; Reynes et al., 2011). Elle indique que chez les animaux séropositifs, les anticorps neutralisants détectés sont le reflet d'une infection ancienne, et que le système immunitaire aurait réussi à éliminer le virus. De fait, de nombreuses études ont montré que l'ARN lyssavirus n'est détecté que chez des individus symptomatiques, présentant des signes cliniques caractéristiques d'une infection neurotrope (déséquilibre, perte d'orientation, agressivité, etc.) (Markotter et al., 2006 ; Aubert, 1999 ; King & Crick, 1988). Il est possible que pour certaines raisons que l'on ignore (perte de fitness de l'hôte ?), chez certains individus, l'équilibre entre le système immunitaire et le niveau de réplication virale serait rompu entraînant la multiplication virale et provoquant l'état morbide caractéristique de la neurovirulence et pouvant même provoquer la mort de l'individu (Kuzmin et al., 2008 ; Kuzmin et al., 2010). Dans d'autres modèles animaux d'infection à Lyssavirus, comme l'infection expérimentale du chien par le virus rabique, tous les intermédiaires peuvent être observés depuis l'infection symptomatique et rapidement mortelle jusqu'à l'infection très lente et progressive sur plusieurs mois en passant par une infection asymptomatique et apparemment contrôlée chez une minorité d'animaux qui survivent (tout en excrétant de façon discontinue le virus dans la salive) (Fekadu, 1988 ; Baer, 2012). Il serait utile de vérifier si l'absence de localisation cérébrale des lyssavirus chez les chauves-souris telle que prouvée dans notre série, est corrélée ou pas à l'absence d'excrétion virale du même virus dans la salive. La dissociation entre les deux tissus serait un argument en faveur de l'établissement d'une persistance virale chez les chiroptères due à un petit pourcentage d'animaux à la fois réservoirs et transmetteurs.

D'une façon générale, les virus doivent se répliquer et se transmettre au-delà du seuil d'extinction ($R_0 > 1$) afin de ne pas disparaitre et ce maintien doit être amélioré continuellement dans le temps sans enclencher des réponses immunitaires inhibitrices, ou entrainer le décès rapide de l'hôte ce qui interromprait prématurément la transmission (Real & Biek, 2007). Il est aussi fort à penser que la persistance virale chez les chauves-souris dans la plupart des études, n'est peut-être pas le reflet de la balance immunitaire de l'hôte, mais plutôt la conséquence d'une stratégie réplicative virale contrôlée, très opportune et très ancienne de survie des virus. Il est aussi envisageable qu'une infection virale persistante puisse apporter une protection contre d'autres agents pathogènes par le biais d'une interférence virale avec réponse d'Interférons de type I endogène. Une telle interférence virale protectrice a été démontrée dans l'infection expérimentale de *Rattus* par le bacille *Yersinia pestis* agent de la peste. L'infection bactérienne, toujours mortelle est totalement neutralisée par une infection latente des rats par un virus herpes murin (Barton et al., 2007).

L'enquête séro-épidémiologique conduite sur les chauves-souris et les petits mammifères terrestres de la région du SOOI utilisant les chips composites pour la détection d'anticorps en IIF contre 15 virus à ARN monocaténaires appartenant à 5 familles virales a apporté des informations intéressantes. Cinq virus représentent la famille des *Paramyxoviridae* : le virus de la rougeole (genre *Morbillivirus*), le *Parainfluenza virus 2* et le virus des oreillons (genre *Rubulavirus*), le RSV (genre *Pneumovirus*), le virus *Parainfluenza virus* 1 (genre *Respirovirus*). Cette étude a permis de révéler que les anticorps anti-Paramyxovirus détectés chez les chauves-souris sont principalement dirigés contre les deux virus appartenant au genre *Rubulavirus* représentés sur la lame chips : oreillons et PIV2 (et beaucoup plus rarement contre le virus de la rougeole, PIV1 et RSV). Il aurait été très intéressant de pouvoir corréler ce résultat sérologique anti-RubulaV avec l'expression d'ARN du genre *Rubulavirus* afin d'éclairer la relation entre présence d'anticorps et persistance virale. Malheureusement, notre système de détection RT-PCR employé pour les paramyxovirus , utilise des oligonucléotides du système RMH, connu pour amplifier des régions fortement conservées du génome dans le gène codant la polymérase (L) des paramyxovirus des genres *Respirovirus*, *Morbilivirus* et *Hénipavirus* (Tong et al., 2008). Ce système ne détecte pas le genre *Rubulavirus*. Un autre système amplifiant les genres, *Pneumovirus, Avulavirus* et *Rubulavirus* (PAR) a été testé au sein de notre équipe de recherche mais il s'est avéré moins sensible et génère de faux positifs.

De façon surprenante, la seule corrélation négative que nous avons pu caractériser dans notre étude sérologique relie la présence des anticorps anti-rubulavirus à l'absence de détection d'ARN *d'URMVs* qui phylogénétiquement sont plutôt classés comme Morbilli-related (*UMRVs*). L'interrogation que pose cette corrélation négative ne pourra être clarifiée qu'une fois notre étude complétée par la détection spécifique des ARN de *Rubulavirus* par le système PAR. En attendant, si elle devait correspondre à une réalité biologique, la corrélation négative indiquerait une antigénicité croisée entre *Rubulavirus* et *UMRVs,* ou la possibilité d'échanges génétiques (recombinaison) entre ces derniers. La séquence complète de quelques *UMRVs* actuellement très avancée au CRVOI permettrait de répondre à ces questions.

Conclusion et perspectives

Nos résultats apportent des informations totalement nouvelles, jamais rapportées jusqu'à aujourd'hui dans la zone SOOI. Les investigations menées au cours de ces 3 années de recherche, grâce aux échantillonnages intenses, aux traitements des échantillons et *in fine* à l'analyse via des outils de bioinformatiques ont apporté un éclaircissement sur la caractérisation et la circulation de virus hautement diversifiés au sein d'une communauté de chauves-souris et de petits mammifères terrestres de la zone SOOI.

Ces déterminants écologiques et génétiques mis en évidence, font partie des critères qu'il convient d'investiguer de façon prioritaire afin de mieux appréhender le risque d'émergence, dans le futur, de ces agents potentiellement zoonotiques dans les populations animales ou humaines (Woolhouse et al., 2005 ; Keusch et al., 2009 ; Morse et al., 2012).

Plusieurs perspectives sont envisageables pour la suite.

Bien que la sérologie RFFIT soit spécifique et indique une circulation intense d'au moins deux lyssavirus appartenant aux phylogroupes I et II respectivement, nous n'avons pas pu identifier les espèces virales en cause. Cela est rendu d'autant plus complexe par la présence de nombreuses réactions croisées en lyssavirus au sein du même phylogroupe. Dans l'objectif de caractériser les déterminants évolutifs et écologiques des lyssavirus et de leurs hôtes dans la zone du SOOI ou encore les classer phylogénétiquement, il serait souhaitable de réaliser un échantillonnage d'individus présentant des symptômes caractéristiques de la rage ou des individus morbides.

La possibilité de *host-switch* d'agents zoonotiques de la population sauvage vers les populations domestiques, constitue l'une des premières étapes avant l'émergence vers les populations humaines (Jones et al., 2008 ; Morrens et al., 2012). Ainsi, dans la continuité de notre étude, il serait intéressant d'investiguer les populations d'animaux domestiques et les populations humaines susceptibles d'être en contact avec les micromammifères des îles

étudiées. Des analyses sérologiques permettront d'évaluer le potentiel risque de sauts d'espèces de ces paramyxovirus de chauves-souris vers ces différentes populations.

Celles-ci pourraient se faire après analyse des séquences complètes de paramyxovirus que nous avons obtenus qui permettra non seulement de classer "définitivement" phylogénétiquement ces nouveaux virus mais aussi de réaliser des tests de sérologie spécifiques afin d'apprécier le taux d'exposition virale antérieure chez ces individus. Ceci étant, nous avons pu réaliser le séquençage complet des paramyxovirus qui ont pu être isolés confirmant que les *UMRVs* appartiennent tous à un même lignage structuré, et proches des *Morbillivirus,* mais ne peuvent pas être considérés comme des *Morbillivirus* : leur structure génomique et la taille de leurs génomes diffèrent - Les *UMRVs* font environ 18 kb contre 15 kb pour les *Morbillivirus* (analyses en cours de finalisation).

Outre *Rattus rattus*, existent-t-ils d'autres réservoirs/vecteurs capables de véhiculer des paramyxovirus génétiquement très proches entre des gîtes de chauves-souris géographiquement très éloignés ? L'équipe de Ci-Xiu Li et al. (2015) a mis en évidence chez plus de 70 espèces d'arthropodes près de 112 nouveaux virus à ARN. Ces virus étaient notamment très proches des arénavirus, filovirus, hantavirus, influenzavirus, lyssavirus et paramyxovirus. Les arthropodes de la zone du SOOI participeraient-ils à la transmission virale au sein de chauves-souris localisées dans des gîtes très éloignés ? Pour répondre à cette question, il serait intéressant de déterminer la richesse virale inféodée aux arthropodes du SOOI.

Il est connu que La Réunion, Maurice et Madagascar hébergent chacune une espèce endémique « sœur » de chiroptère, respectivement, *Mormopterus francoismoutoui* (La Réunion)*, Mormopterus acetabulosus* (Maurice) et *Mormopterus jugularis* (Madagascar). Sur la base d'éléments moléculaires et morphologiques, *Mormopterus acetabulosus*, qui était jusqu'à présent considérée comme endémique des îles Mascareignes (Maurice et La Réunion), a été subdivisée très récemment en deux taxons étroitement liés, *Mormopterus acetabulosus* et *Mormopterus francoismoutoui* : les deux groupes étant réciproquement monophylétiques, mais séparés par une moyenne de divergence de l'ordre de 5% (Goodman et al., 2008). Par ailleurs, ces deux espèces sont également proches de *Mormopterus jugularis*. Il serait intéressant de déterminer si les virus au sein de ces chauves-souris vivant sur 3 îles différentes avec des écosystèmes contrastés répondraient aux mêmes déterminants génétique

et écologique, au regard de la récente radiation observée. Serait-il possible d'observer cette fois-ci une co-divergence virale associée à de la co-spéciation ? Quels sont les facteurs écologiques dans les 3 îles, favorables à la transmission virale ?

Finalement, il serait utile de déterminer si les infections des chauves-souris par des virus, bactéries ou parasites les protégeraient contre l'une ou l'autre de ces infections ou contre d'autres pathogènes dans le cadre d'une pathocénose infectieuse. Pour répondre à cette question, nous analyserons en collaboration avec Yann Gomard et Beza Ramasindrazana, l'ensemble des résultats générés sur le même échantillonnage de chauves-souris dans les 3 modèles biologiques explorés au CRVOI : à savoir le modèle viral des paramyxovirus, le modèle bactérien de la leptospirose, et le modèle parasitaire des hémoparasitoses.

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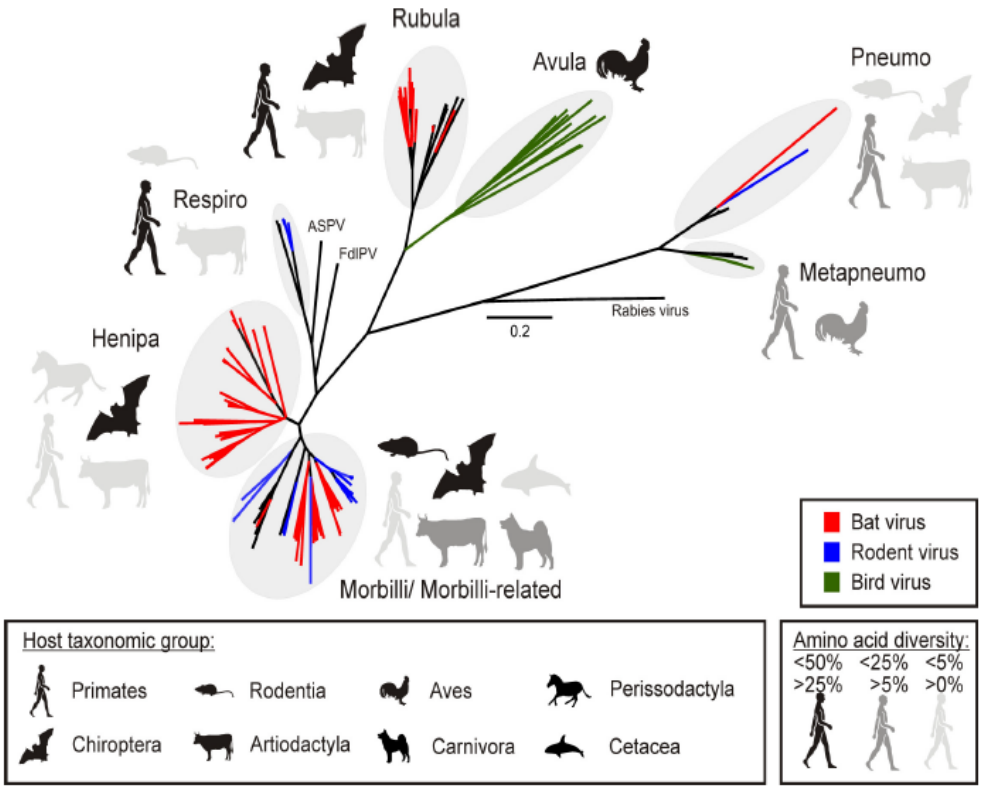
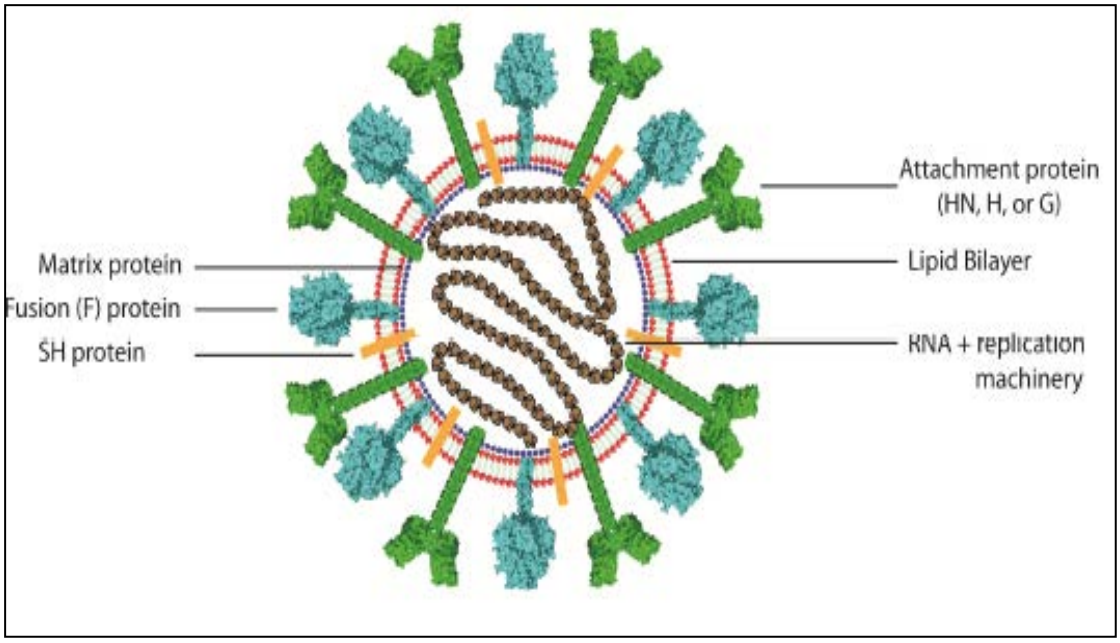
Annexes

Annexe 1. Phylogénie de la famille des *Paramyxoviridae* (Drexler et al., 2012).



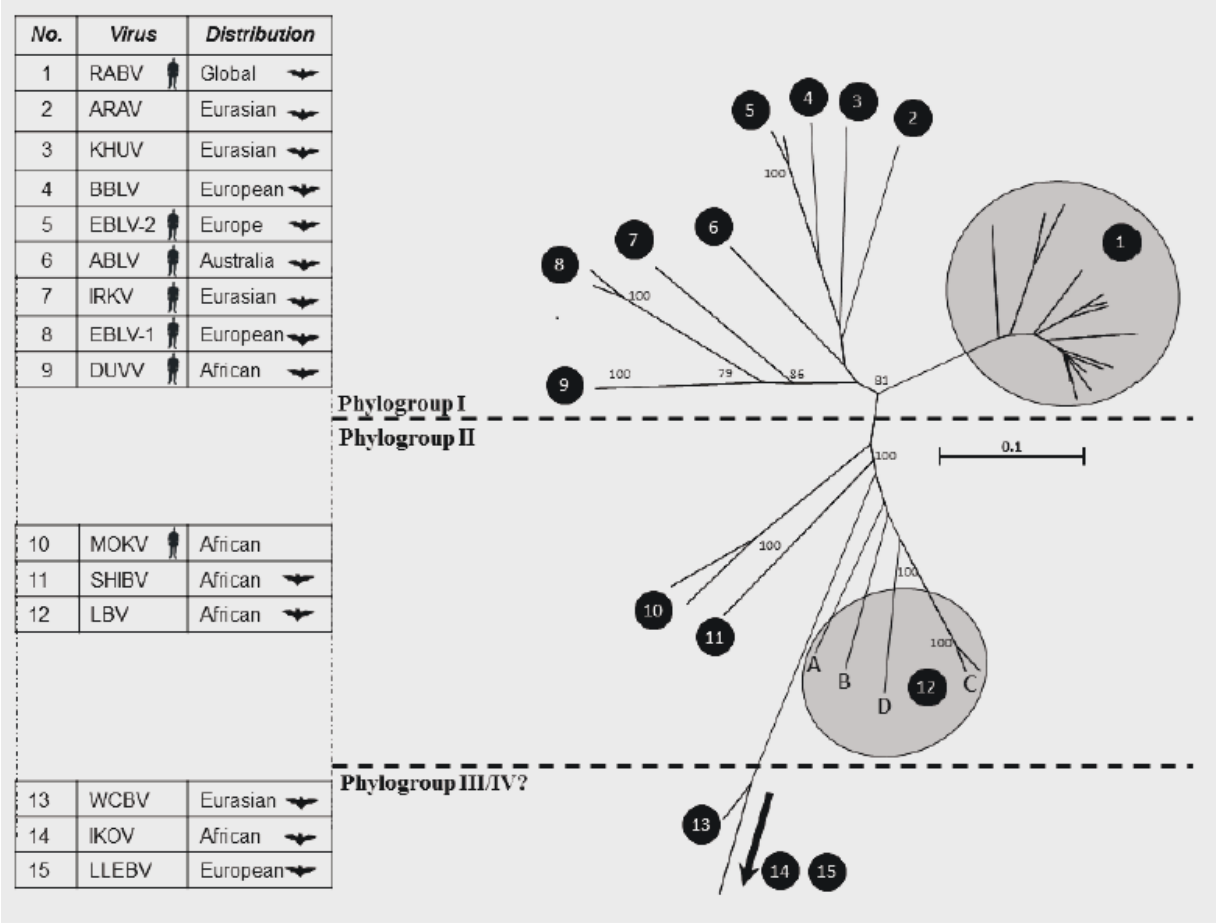
Annexe 2. Structure schématique d'un paramyxovirus

(Chang et al., 2012).

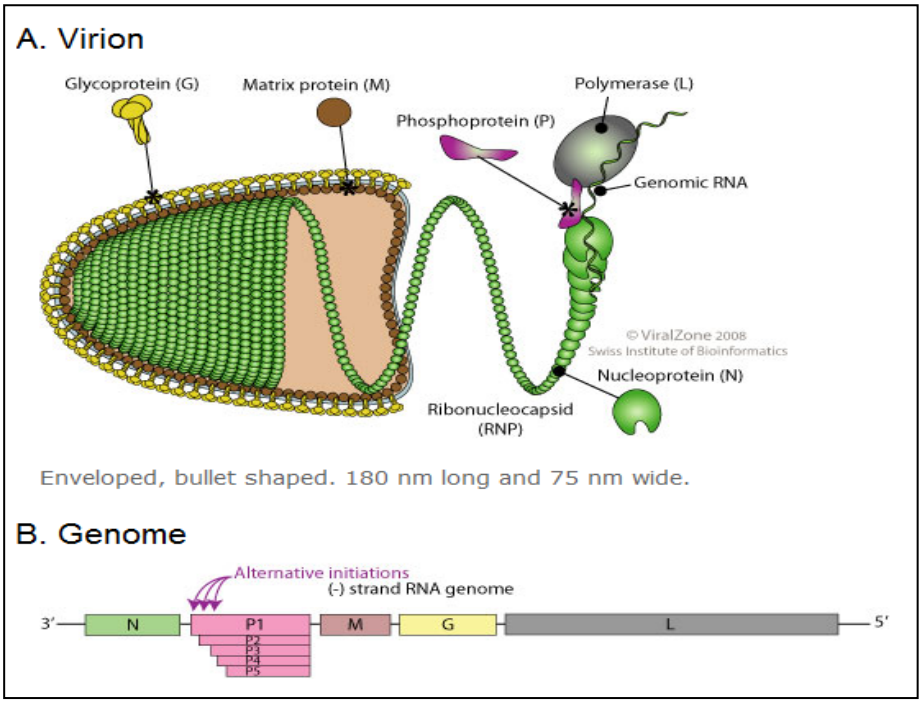


Annexe 3. Phylogénie et distribution géographique des lyssavirus

(Banyard et al., 2014).



Annexe 4. Structure générale (A) d'un virion et (B) d'un génome d'un Rhabdovirus (Source : Viralezone)



Annexe 5. Insight into the global evolution of Rodentia associated Morbillirelated Paramyxoviruses reveals the role of *Rattus* as a worldwide spreader (soumis)

Insight Into the Global Evolution of *Rodentia* Associated *Morbillirelated* Paramyxoviruses Reveals the Role of *Rattus* As A Worldwide Spreader

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Running Head: *Rodentia* Associated *Morbillirelated* Paramyxoviruses

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W.G. and H.P. contributed equally to this work.

Words count: abstract (196) and text (6674).

Abstract

Paramyxoviruses (PVs) are a large and diverse family of viruses that affect humans and a wide range of animal species. Among this family, a group of *Unclassified Morbilli-Related Viruses* (*UMRVs*) has been recently recognized in wild small mammals, but its evolutionary history is still poorly understood. In the present study, 160 rodents belonging to four species, indigenous to Northern Africa, namely *Psammomys obesus*, *Ctenodactylus gundi*, and *Meriones shawi,* as well as the cosmopolitan *Rattus* (*R.*) *rattus* were captured in Tunisia and analyzed for PV infection using RT-PCR targeting part of the L-polymerase gene. PVs were detected in 49 out of 160 rodents yielding a global positivity rate of 30.6% and analyses allowed their identification as novel *Rodentia UMRVs*. Phylogenetic and phylogeographic analyses were conducted based on the *URMVs* sequences amplified from positive animals together with *URMVs* sequences from rodents originating in all continents and recovered from public databases. These analyses revealed three major different clusters among *Rodentia*-associated *UMRVs* and suggest that strain diversification occurred during geographic migration and host exchanges with purification selection pressure as the principal evolving force. *Rodentia*-associated *UMRVs* likely originated in Southern Africa and were mainly vectored by *R. rattus* to emerge worldwide.

Importance

Recently, novel Paramyxoviruses known as *Unclassified Morbilli-Related Viruses* (*UMRVs*, *Paramyxovirinae*, *Paramyxoviridae*) were reported to infect at high infection rates, various wild small mammal orders. The current study shows those novel *UMRVs* are endemic to four wild rodent species captured in the Center and the South of Tunisia. The highlighting of the history of these *Rodentia* associated *UMRVs* was made possible because we compared endemic species to highly localized species in North Africa. The genetic diversity detected among the *Rodentia*

48 associated *UMRVs* and their phylogeography contributes to the understanding of their molecular
49 epidemiology and their natural evolutionary history. The study highlights and confirms the role
50 of *Rattus rattus* in the global dispersal of the *UMRVs*.
51 Our findings provide important knowledge on the epidemiology of the *UMRVs* and might help
52 understanding why they still worldwide spreading.
53

54 ## Introduction

55 *Rodentia* is the most species rich mammalian order and has been found to carry the highest
56 number of zoonotic pathogens (1). Hence, eco-epidemiological studies on rodents as reservoirs of
57 pathogens are highly relevant to human health (2, 3) and to the design of control programs of
58 rodent-borne diseases.
59 *Rattus* (*R.*) *rattus* is by far the most important invasive rodent and reservoir host which vectors
60 dissemination of many pathogens globally (4, 5). This species has an Asian origin which dates
61 back to over one million years ago (middle Pleistocene age) as assessed by molecular clock
62 estimates (6) and the detection of fossils in Thailand(7, 8) and Java(9). Then, this species had a
63 global dispersal bringing significant implications to human health and economy.
64 Paramyxoviruses (PVs) are a family of negative-sense single-stranded RNA viruses that
65 infect a large range of hosts including mammals, birds, reptiles and fish (10). The group includes
66 established pathogens of humans (e.g. *Measles* and *Mumps* viruses), rodents (e.g. *Sendai* virus),
67 avifauna (e.g. *Newcastle Disease* virus), livestock (e.g. *Rinderpest* virus), canids (e.g. *Canine*
68 *Distemper* virus) and also new emerging zoonotic viruses of medical importance such as *Hendra*
69 or *Nipah* viruses (10, 11). Some other PVs may have significant socio-economic impact such as
70 the equine disease outbreak caused by *Salem* virus (12). Recently, novel PVs known as

71 *Unclassified Morbilli-Related Viruses* (*UMRVs, Paramyxovirinae, Paramyxoviridae*) were
72 reported to infect at high infection rates, various wild small mammal orders (*Rodentia,*
73 *Afrosoricidae, Chiroptera*) worldwide (13, 14). *UMRVs* are phylogenetically closely-related (14)
74 but their molecular evolutionary history is still poorly understood. Heretofore, few *UMRVs* have
75 been reported to be pathogenic to the animal host, such as the *J* and *Belinga* viruses inducing
76 hemorrhagic lesions in rodents and bats respectively (15, 16), but, for most of them, there is a
77 lack of information regarding their pathogenicity. Virus transmission between animals belonging
78 to the same species is mainly horizontal suggesting that contaminated feces, urine or saliva are
79 potentially contagious to other species including humans, living in contact (17).
80 In this study, we investigated the phylogeography and the evolutionary history of *UMRVs*
81 among reservoir species belonging to the order *Rodentia* and the role played by the *R. rattus* in
82 the global dissemination of these viruses. Our study was focused on PVs infecting rodents from
83 Tunisia: three wildlife species, namely, *Psammomys* (*P.*) *obesus*, *Meriones* (*M.*) *shawi*, and
84 *Ctenodactylus* (*C.*) *gundi,* all indigenous to the North African countries, and one cosmopolitan
85 species, *R. rattus*. Then, using phylogenetic and phylogeographic tools , we compared sequences
86 derived from these Tunisian rodents to those reported in public databases originating from
87 rodents distributed worldwide, to identify the relationship between *UMRVs*, their geographic
88 origins and their animal host reservoirs and to infer their origin as well as the mechanisms of their
89 global evolution.
90

91 ## Materials and Methods

92 **Ethics Statement**

93 The research protocol was approved by the IRB at Institut Pasteur de Tunis. All animal
94 experimentations in the field to collect animal samples and individual data on captured animals,
95 were conducted in Tunisia, and were in agreement with the guidelines of International Guiding
96 Principles for Biomedical Research Involving Animals. Study of Infection and analysis were
97 performed at Centre de Recherche et de Veille sur les maladies Emergentes dans l'Océan Indien,
98 La Réunion, France.

99 **Sampling of rodents in Tunisia**

100 Between 2009 and 2013, 784 wild rodents were trapped as previously described (18) in five
101 districts belonging to two governorates of Tunisia: the districts of Mnara, Khbina and Ouled
102 Mhamed were located in the governorate of Sidi Bouzid in the subhumid central Tunisia, and the
103 districts of Germessa and Mdhila were located in the governorate of Tataouine in the arid
104 southern Tunisia (Fig. 1). The sites of capture were geo-referenced by GPS and trapped rodents
105 were transferred alive to the laboratory for physical examination and tissue sampling. Relevant
106 parameters were recorded for each animal: species identification, sex and standard morphometric
107 measurements. After cardiac exsanguination, small tissue fragments were cut from heart, liver,
108 kidney, spleen, lungs and brain organs; snap frozen in liquid nitrogen and stored in deep freezer
109 at -80°C until analysis. Rodent age was estimated by measuring the lenses weight as previously
110 described (18).

111 Pearson Chi-Square and Fisher exact tests allowed comparison of categorical variables.
112 STATA software version 11 was used to carry out all statistical analysis.

113 **Paramyxoviruses detection by RT-PCR**

114 For each animal, total nucleic acids were extracted from approximately 1 mm^3 of lungs, kidneys
115 and spleen using the viral mini kit v2.0 and an EZ1 BioRobot (QIAGEN). Tissues were
116 homogenized in DMEM medium by TissueLyser (QIAGEN) for 2 min at 25 Hz using 3 mm

117 tungsten beads. cDNA was generated through reverse transcription (Promega cDNA kit). Semi-
118 nested PCR targeting a partial sequence of the L-gene polymerase locus (~490 bp) of
119 *Paramyxovirinae* subfamilies was carried out using previously described conditions (14, 19). RT-
120 PCR products were purified using the QIAquick PCR purification kit (QIAGEN), cloned into the
121 pGEMt-Easy vector (Promega), according to the manufacturer's instructions and sequenced using
122 chain-termination (Sanger) sequencing (Big Dye Sequencing kit, ABI) on both strands by a
123 commercial service (Genoscreen, Lille, France). Forty-four viral sequences from rodents caught
124 in Tunisia were generated in the present study and were deposited in Genbank with the reference
125 numbers: KJ408384 – KJ408428.

126 **Origin of the viruses and data sets construction**

127 Phylogenetic and phylogeographic analyses were conducted using the 42 partial L-gene *UMRVs*
128 sequences from rodents caught in Tunisia into a panel of 145 partial *UMRVs* sequences infecting
129 rodents from over 11 countries, recovered from public databases spanning years 1962 to 2013.
130 Only sequences with well-documented collection date and location origin were considered for
131 analysis. Three data sets were used: the first set (Supplementary Table S1 in Supplementary
132 material) was representative of most of PVs genera and allowed classification of Tunisian
133 sequences among *Paramyxoviridae* family, the second set (Supplementary Table S2 in
134 Supplementary material) was devoted to the phylogenetic and the phylogeographic analysis of
135 the *Rodentia* associated *UMRVs*. The third data set recovered from Genbank, was focused only
136 on *Measles* virus (MeV) (Supplementary Table S3 in Supplementary material) and was used to
137 estimate the evolutionary rate of the L-gene polymerase locus.

138 **Phylogenetic analysis: maximum-likelihood and Bayesian inference analysis**

139 Partial sequences of the polymerase of PVs infecting rodents from Tunisia were compared to
140 published sequences in GenBank (National Center for Biotechnology Information, Bethesda,

141 USA) online (www.ncbi.nih.gov) using BLASTn and BLASTx. Raw sequences were then
142 cleaned for primers sequences, edited, assembled and compared using Geneious®Pro 7.1.8 (20).
143 The multiple alignments of partial L-gene sequences were performed with MAAFT 7.0.17
144 algorithm (21).
145 A GTR nucleotide substitution model with rate variation and a proportion of invariant sites
146 (Gamma + I) was determined to best fit the data using Akaike Information Criterion in MEGA6
147 (22), and in ModelTest via the integrated Geneious Paup tool (23). The same best-fit substitution
148 model was used for all phylogenetic analyses. Phylogenetic relationships among PVs strains
149 based on partial L-gene sequences were analyzed using both Bayesian inference and maximum
150 likelihood (ML) methodologies. ML trees were estimated, with 1000 rapid bootstraps, using
151 RAxML 7.2.8 Geneious plugin, under the best-scoring ML tree parameter, and using Mega6 (22).
152 Bayesian phylogenetic trees were supported by the topology of the ML trees (data not shown).
153 Bayesian inference analysis were performed with the MrBayes 3.2.2 plugin included in Geneious
154 software (24), with constrained and unconstrained branch lengths, four Metropolis-coupled
155 chains for 10 million Markov Chain Monte Carlo (MCMC) generations, sampling of the Markov
156 chain every 200 generations, discarding the 25% as burn-in. The sequences of bat-rabies virus
157 (JQ595353) and rodents PVs (RodPVs) (HQ660195 and AB844426, 2 *Respirovirus* sequences)
158 were chosen as outgroup in Fig. 1 and 2, respectively. Trees were visualized by FigTree 1.4.2
159 (www.tree.bio.ed.ac.uk/software/figtree/). The applicability of a molecular clock to the data set
160 was evaluated with a log-likelihood-ratio test using the ML scores, with and without a molecular
161 clock enforced. The null hypothesis of equal evolutionary rate throughout the trees was not
162 rejected at a 5% significance level when assessed using Mega6 (22). As a consequence, all
163 subsequent phylogenetic analyses were performed using a strict-molecular clock. For the
164 Bayesian tree reconstructions no difference was observed between strict- and relaxed-molecular

clocks. However, for the phylogenetic analysis performed with Beast package 1.8.2 (25), we used a relaxed-clock Bayesian MCMC method, as previous studies have shown that it allowed a better fit to the data than a strict clock (25-27).

Bayesian evolutionary analyses of spatio-temporal *UMRVs* dynamics

To explore the evolutionary relationships and times to the most recent common ancestors (t_{MRCAs}) among *UMRV* lineages circulating at a global level, we reconstructed phylogenetic history using Bayesian MCMC analysis implemented in the Beast package. *UMRVs* sequences were annotated to one of 11 locations accordingly to the country of origin (Australia, China, Germany, Madagascar, Mayotte, Reunion, Seychelles, South Africa, Trinidad & Tobago, Tunisia and Zambia), and used as a discrete trait during MCMC analysis. We employed a GTR + G + I model of nucleotide substitution with a normally distributed rate variation among sites (3.48×10^{-4}, sd 1.69×10^{-5}, values obtained from the MeV data set analysis (See Supplementary Text 1 and Supplementary Table S3 in Supplementary material)) and a relaxed (uncorrelated log-normal) molecular clock model. First, we specified a Bayesian skyline population coalescent model (10 piece-wise constant groups) (26), then, we investigated, with the same prior distribution, a non-parametric skygrid plot model (28). All chains were run for a good enough length and convergence was assessed using Tracer, with statistical uncertainty reflected in values of the 95% highest posterior density, removing at least 10% of the chain as burn-in. The probable locations of each ancestral node and evolutionary time past were summarized using an annotated Maximum Clade Credibility (MCC) phylogenetic tree. Posterior probability values provide an assessment of the degree of support for each node on the tree.

Lemey et al. (29) have implemented a continuous-time Markov chain (CTMC) over discrete sampling locations in the Beast package, making it possible to model spatial diffusion on

188 a time-scaled phylogenetic tree (29). To determine the different lineage migration patterns,
189 inferred with a Bayesian stochastic search variable selection scheme (BSSVS), allowing the
190 switch rates in the CTMC to be zero with some prior probability, we used either a CTMC
191 symmetric (reversible: bidirectional between locations) or asymmetric (non-reversible:
192 unidirectional route) substitution models for discrete geographic traits. Coalescent models were
193 compared using a modified Akaike information criterion (AICM) in Tracer (30). The BSSVS
194 procedure computes the most parsimonious possible rate values, by assigning a Bayes factor (BF)
195 significance test between transmission routes, explaining the different forms of diffusion. The
196 result has been calculated and visualized by Spread (31), with a BF cutoff greater than 3 taken as
197 significant support, as previously reported (29, 32).

198 **Selection pressure**

199 First, we used a series of tests provided by MEGA6 (22). We conducted Tajima's test of
200 neutrality, a statistical test to compare the number of segregating sites per site with the nucleotide
201 diversity. The purpose of the test is to determine between sequences randomly evolving
202 (neutrally) and those whose evolution is driven by a non-stochastic process (directional selection,
203 balancing selection, demographic expansion or contraction, etc.). A Tajima's D = 0, means that
204 the population dynamic evolves without evidence of selection. When D < 0, it means that
205 population size is not at equilibrium, but expanding, due to an over representation of infrequent
206 polymorphisms, typical after a bottleneck or a selective sweep and/or a purifying selection. On
207 the opposite, if D > 0, it represents a decrease in population size and/or balancing selection due to
208 an under representation of both low and high frequency polymorphisms. Based on the
209 comparison of the numbers of Synonymous (d_S) and Nonsynonymous (d_N) substitutions between
210 sequences, a codon-based Fisher's exact test has been conducted, and also an Estimate Selection
211 for each codon (HyPhy). The latter computes the selection robustness, whether negative or

positive, acting on each of the corresponding codon and provides statistical estimates. Considering the rather large size of our dataset, we made also a Z-Test to test the null hypothesis that H_0: $d_N = d_S$. The level of significance at which H_0 is rejected depends on the respective alternative hypotheses (HA): (a), (b), or (c) with (a) $d_N \neq d_S$ (test of neutrality), (b) $d_N > d_S$ (positive selection), (c) $d_N < d_S$ (purifying selection). Selection pressures were also measured using SLAC/REL/FEL maximum likelihood methods, MEME, FUBAR, BGM, and DEPS/FADE via the Datamonkey facility (33). The recombination detection program (RDP) implemented in the RDP 4.46 software package (34) was also used.

Accession numbers generated in the present study

Forty-four viral sequences of *UMRV* from rodents caught in Tunisia were generated in the present study and were deposited in Genbank with the reference numbers: KJ408384–KJ408428.

Results

Rodents belonging to four species namely *P. obesus* (*Muridae*), *M. shawi* (*Muridae*), *R. rattus* (*Muridae*) and *C. gundi* (*Ctenodactylidae*) were randomly selected (40 individuals per species) from a large panel of animals captured in Tunisia and tested by RT-PCR for infection with PVs (Table 1). PVs RNAs were detected in 49 out of 160 rodents yielding a global positivity rate of 30.6% (range 2.5 - 50% according to the rodent-species ($p < 10^{-3}$) (Table 2). There were no significant differences in the infection rates according to sex, morphometric parameters, age and sampling site location.

PVs infecting wild rodents from Tunisia are *UMRVs*

Partial L-gene sequences of the PVs were obtained from 45 PVs positive rodents from Tunisia (designated hereafter as RodPVs-Tun). In order to correctly assess their evolutionary position, we

235 performed a Bayesian phylogenetic analysis representing most of PVs genera. As shown in Fig.
236 1, RodPVs-Tun shape a strongly supported sister clade along with morbilliviruses (BP = 0.98)
237 allowing to identify them as *UMRVs*.

238 RodPVs-Tun can be further subdivided into two discrete phylogroups (BP = 0.92). The
239 first phylogroup shapes a clade that gathers all RodPVs-Tun hosted by *P. obesus* and most *C.*
240 *gundi*, as well as the unique sequence from *M. shawi*. On a basal position to this clade, are the
241 *Mossman* and *Tupaia* viruses (35, 36) as well as *Salem* virus (12). The second phylogroup gathers
242 the rest of the RodPVs-Tun, i.e., all those detected in *R. rattus* and some PVs sequences from *C.*
243 *gundi*, together with *Tailam*, *Beilong* and *J* viruses (15, 37, 38). In contrast to the phylogroup 1,
244 where *P. obesus* and *C. gundi* clustered separately into two rather well-supported subclades (BP
245 = 0.67 and 1 respectively), phylogroup 2 mixed together PVs sequences from *C. gundi* and *R.*
246 *rattus*.

247 Altogether our sampling reveals that at least two main PVs lineages are co-circulating among
248 rodents from Tunisia: *C. gundi* are infected by both lineages whereas *P. obesus*, *M. shawi* and *R.*
249 *rattus* are apparently affected by only one. Interestingly, the viral lineage which infects *C. gundi*
250 and *R. rattus* was found only in these rodents (despite the presence of some other species
251 captured in the same region) and only from *C. gundi* trapped in the governorate of Sidi Bouzid.

252 **Phylogeny of *Rodentia* associated *UMRVs***

253 Using a larger set of data available in GenBank, we then extended the phylogenetic analysis of
254 RodPVs-Tun, to *UMRVs* hosted by rodents originating from various countries, worldwide.
255 Rodents belonged to four families (*Nesomyidae*, *Ctenodactylidae*, *Cricetidae* and *Muridae)* and
256 originated from eleven countries distributed on five continents i.e., Africa (Tunisia, Zambia,
257 South Africa), America (Trinidad & Tobago), Asia (China), Australia, and Europe (Germany), as
258 well as multiple islands from the Southwestern Indian Ocean (SWIO) region i.e., Madagascar,

Mayotte, Reunion, Seychelles. Noteworthy, a bias in sample representativeness, inherent to sequence availability in GenBank, is introduced by the fact that *Muridae* family included more than ten different species. The topology of the tree defines, at the global level, four relatively well-supported major paraphyletic clades (Fig. 2; BP = 0.78, 0.93, 1 and 1, respectively for clades I, II, III and IV). Each clade grouped *UMRVs* that were hosted by rodents belonging to various species and originating from distant countries (Fig. 2, branches of the phylogenetic tree are colored according to the geographic origins of the *UMRVs*).

Hence, Clade I includes *UMRVs* sequences infecting *C. gundi* and *R. rattus* collected in Tunisia together with sequences from *Eliurus* (*E.*) *sp.* and *Rattus sp.* from Madagascar and SWIO Islands, respectively. Similarly, *UMRVs* sequences from *Rhabdomys* (*R.*) *pumilio*, *Mastomys* (*M.*) *natalensis*, *Apodemus* (*A.*) *flavicollis*, *Myodes glareolus* and *Microtus (M.) sp.* from Germany, South Africa and Zambia also clustered in this clade I, together with sequences of *Jeilong* virus group (*Rattus sp.* group 2). *Tailam* and *Beilong* viruses are in basal position of a group comprising only *UMRVs* sequences from *Rattus sp.* from some SWIO Islands, and *J* virus is inserted between *M. natalensis* group 1 and sequences from *A. flavicollis* group. Clade II includes *UMRVs* sequences from *P. obesus* from Tunisia shaping a phyletic group associated with *Rattus sp.* sequences from the SWIO Islands (*Rattus sp.* group 3), *M. natalensis* group 2 from Zambia clustered with one *R. pumilio* sequence from South Africa. Most sequences isolated from *C. gundi* clustered with the only sequence from *M. shawi* and with a Malagasy *R. rattus* sequence (*C. gundi* group 2). This latter is sister group with another mixing group from Madagascar (*E. minor* group 2 and *Rattus sp.* group 3). Besides, clade II holds in a separate cluster two Zambian sequences of *Mus (M.) Minutoides* and one *Rattus* sequence from Reunion Island. Clade III brings together *Mossman* and *Nariva* viruses and *M. natalensis* group 3 from Zambia while the South African *R. pumilio* group 2 corresponds to Clade IV.

283 Obviously, the evolutionary history of *UMRVs* cannot be explained only on the basis of a
284 geographical structuring. For example, *UMRVs* from Tunisia were genetically strongly related to
285 *UMRVs* from SWIO Islands. In both clades I and II, closely related PVs infect *C. gundi*
286 indigenous to Northern Africa and *Eliurus sp.* endemic to Madagascar. The same holds true for
287 *UMRVs* circulating in Germany (*A. flavicolis*) and in Zambia (*M. natalensis*).
288 However, the topology of the tree reveals some host virus-species associations; for instance
289 *UMRVs* infecting *Rattus sp.* from the SWIO Islands define 3 groups clustered into two well-
290 supported lineages; Similarly, *UMRVs* infecting *R. pumilio* from South Africa feature 2 groups
291 clustered into two well-supported lineages; *UMRVs* infecting *M. natalensis* from Zambia, 3
292 groups clustered into three well-supported lineages; *A. flavicollis*, *M. glareolus* and *Microtus sp.*
293 from Germany (each rodents-species with one group) and *P. obesus* from Tunisia (one group).
294 Nonetheless, the evolutionary history of *UMRVs* infecting *Rodentia* suggests the occurrence of
295 different events. That closely structured *UMRVs* sequences are shared between different rodent
296 species, geographically close or distant, suggests the occurrence of host-jumps or/and spatial
297 diffusion. The fact that most of them involve *Rattus sp.* as a partner (represented by an asterisk in
298 Fig. 2), pinpoints the possible role of this species as diffusing vector. Thus, *UMRVs* from *C.*
299 *gundi* from Tunisia illustrate a species jump with *R. rattus* from Tunisia in clade I and another
300 jump with *M. shawi* from Tunisia and *R. rattus* from Madagascar in clade II. In both cases *C.*
301 *gundi* sequences were closely structured with those harbored by *E. minor* from Madagascar
302 which presents a spillover with *R. rattus* from the same Island.
303 **Phylogeography analysis of *Rodentia* associated *UMRVs***
304 Fig. 3 shows the generated Maximum Clade Credibility (MCC) tree (the branches of the tree
305 have been colored according to the most probable location of their descendent nodes). Two main
306 clades are segregated: clade A and clade C.

307 Clade C includes five *UMRVs* sequences harbored by *R. pumilio* from South Africa whereas
308 clade A is resolved into two major Subclades: subclade A1 groups sequences distributed
309 worldwide and subclade B includes only African sequences significantly segregating into four
310 distinct subgroups: Tunisia (B2); South Africa (B3); Zambia (B4) and some SWIO Islands i.e.,
311 Mayotte, Reunion and Madagascar (B5). Subclade A1 contains two major phylogroups: A2 and
312 A'2. The latter groups two Zambian sequences clustered with Tunisian and Malagasy sequences
313 (A'3) as well as with *Nariva* and *Mossman* viruses, originating from Trinidad & Tobago and
314 Australia, respectively. In fact, most *UMRVs* sequences infecting *C. gundi* from Tunisia clustered
315 with one sequence isolated among *R. rattus* from Madagascar (A'5) that significantly segregated
316 from Malagasy sequences from *E. minor* and *R. rattus* (A'4).
317 Phylogroup A2 segregated into several subclades (A3-A9): The remaining *UMRVs* sequences
318 identified in *C. gundi* from Tunisia were clustered with sequences hosted by *E. minor* from
319 Madagascar and *Rattus sp.* from SWIO Islands (A9). The latter significantly segregated from
320 another clade (A7), contains sequences of *Rattus* sp. from Reunion, Mayotte and Seychelles
321 clustered with *Beilong* and *Tailam* viruses from China. These sequences from Tunisia and SWIO
322 Islands were significantly clustered with sequences from South Africa isolated from *R. pumilio*
323 (A6). *J* virus significantly clustered with four sequences, two of them were from Germany and
324 the two other from Zambia, isolated respectively from *M. musculus*, *A. flavicollis* and *M.*
325 *natalensis* were significantly assigned with the clade A6 forming the clade A5. *UMRVs*
326 sequences from Germany identified in different rodents-species such as *M. glareolus* (forming a
327 monophyletic specific cluster), *M. arvalis*, *M. agrestis* and *A. flavicollis* were significantly
328 clustered with the clade A5 within the clade A4. The clade A3, consisting in two sequences from
329 Zambia and a sequence from Reunion, was clustered with the clade A4 forming the second major
330 phylogroup A2.

Statistical analysis of the phylogeographic tree showed that it most probably rooted in South Africa, with a posterior probability of 1.0. Zambia was also the most probable location of the two main clades A1 and B, and of the two major phylogroups A2 and A'2. Hence, *Rodentia* associated *UMRVs* sequences most probably originated in Africa and their currently circulating sequences have emerged from a common ancestor more than 4000 years ago. Until 2500 years before present, *Rodentia* associated *UMRVs* could be detected only in two African countries: South Africa and Zambia.

The most recent common ancestor (t_{MRCAs}) of all *Rodentia* associated *UMRVs* from Australia and Trinidad & Tobago has most probably emerged 3000 years ago (clade A1; 95% HPD = 2121-3897) whereas European *Rodentia-UMRVs* (represented by Germany) emerged more recently (clade A4; 95% HPD = 1524-2794), and Asian (represented by China) was the last to diverge (clade A7; 95% HPD = 785-1563). However it should be noted that the current circulating *UMRVs* on SWIO Islands and Tunisia have a recent emergence, which does not exceed 200 years ago with the exception of other previous introductions in Reunion Island and Madagascar dating back to around 2100 (clade A3; 95% HPD= 1331-2951) and 1200 (clade A'4; 95% HPD= 779-1628) years ago, respectively.

Bayesian phylogeographic analysis showed 46 well-supported route linkages between countries. We used a Bayes factor (BF) cutoff of 3.0 to determine significance (Supplementary Table S4 in Supplementary material). Most roots involved African countries and SWIO Islands, suggesting that they played an important role in the migration of rodent associated *UMRVs*. The root with the strongest support was found between Zambia and Trinidad & Tobago with a BF of 207. Most roots begun with African Countries (17 with 8 roots begin with South Africa) followed by SWIO Islands (15 roots), Trinidad & Tobago (7 roots), Australia (4 roots) and Germany (3 roots). On the other hand, China was found on 10 roots but only in the end. The map with the

355 rates of transitions constructed using Spread is shown in Supplementary Fig. S1 (Supplementary
356 material).

357 Analysis of the skygrid plot (Fig. 4a) showed that the effective number of *Rodentia*
358 *UMRVs* infections at global level increased rapidly until around 1000 years ago. A further sharp
359 increase in the number of infections occurred between 1000 and 600 years ago when it reached
360 the maximum peak, after which the epidemic growth in diversity stopped and even decreased
361 though it remained at a level higher than it was at the beginning.

362 The effective number of viral lineages depicted in Fig. 4b shows an increasing diversification of
363 *UMRVs* strands over time through three well-defined successive phases. The first one, set
364 between the remote ancestor and the 11th century, and the second one, comprised between the
365 11th century and 1900, are indicative of a linear relationship of diversification process over the
366 time, nonetheless with a faster process regarding the first phase. The third and last phase, starting
367 at 1900 to present, is clearly indicative of a nonlinear process over time, and shows a double
368 increase of the whole diversification process in less than one hundred years.

369 **Selection pressures**

370 The selection pressure operating in the *Rodentia UMRVs* studied here, have been estimated by
371 different statistical methods provided by MEGA6 (data available upon request). Overall, these
372 analyses revealed that purification selection is acting as the principal evolving force, with an
373 abundance of negatively selected sites versus a little support for positive selection. Moreover, we
374 computed different codon-specific substitution likelihood-based methods (Table 3) that
375 accounted for discrete individual codon sites compared to *Peste-des-petits-ruminants* virus
376 (PPRV), *Measles* virus (MeV), Canine distemper virus (CDV), and *Feline morbillivirus* (FMV)
377 for the same L-gene region (33).

Codon-specific-analyses showed that none of the studied viruses showed evidence of positive selection (Table 3), except for *UMRVs* where only three positively selected sites have been inferred with the Mixed Effects Model Evolution (MEME) at a significance value of $p < 0.1$, and just one at $p < 0.05$. The latter is most appropriate to detect episodic diversifying selection affecting discrete codon sites. Interestingly, with DEPS method using amino acid sequences to identify directional evolution towards residues at sites, and valuable for the detection of selective sweeps, there is strong evidence that some sites are evolving under a directional selection in *UMRVs*, but probably not for the considered morbilliviruses, despite the small number of sequences used for testing. Indeed, no evidence of recombination has been detected among those viruses.

Discussion

The infection rate with PVs in four wild rodent-species (*P. obesus*, *M. shawi*, *C. gundi* and *R. rattus*) captured within their natural biotopes in central and southern Tunisia was estimated at 30.6% with the highest rates found in *P. obesus* (50%) and *C. gundi* (47.5%). These rates are to our knowledge, the highest reported so far in rodents (13, 14, 39) other than prevalence of 68% recently reported in the red and gray squirrels in the United Kingdom (40).

Phylogenetic analysis shows that RodPVs-Tun sequences belong to *UMRVs*, as previously reported in other small mammal species (13, 14, 39) confirming the worldwide distribution of these viruses. Until recently, the *Rodentia* taxa harboring *UMRVs* spanned four families *Muridae*, *Cricetidae*, *Sciuridae* and *Nesomyidae* (13, 14, 39, 40). The present study enlarges this range to rodents of the *Ctenodactylidae* family (*C. gundi*) and adds two species of the *Muridae* family (*P. obesus* and *M. shawi*) as potential reservoir hosts.

401 Phylogenetically, RodPVs-Tun fell into two major clades. The first clade contains all
402 sequences from *P. obesus*, *M. shawi* and most sequences from *C. gundi,* while the second clade
403 includes sequences from *R. rattus* and some sequences from *C. gundi*. In fact, the two viral
404 lineages affect in different ways the four rodent-species. The sedentary behavior of *P. obesus*
405 which lives in special ecologic niches i.e. salty biotopes (18), may account for the restricted
406 specificity of the viral strain to this species, in contrast to the other rodent species that show
407 evidence of virus sharing. However, this species restriction may also just reflect a sampling bias
408 and a larger investigation of *R. rattus* trapped in vicinity of *P. obesus* should help clarifying this
409 issue. These sequences were closely-related to *Beilong* and *J* viruses. As the *Jeilong* viruses have
410 been shown to have interchangable genome replication machineries (41), the same feature might
411 also hold true for *UMRVs* that belong to the same phylogroup; however full genome sequencing
412 are needed to ascertain this point.
413 At the global level, *UMRVs* are phylogenetically resolved into three major lineages: one is
414 restricted to viruses from African countries including the SWIO Islands and the two others
415 include *UMRVs* that are distributed worldwide. Clustering of viruses originating from distant
416 locations i.e., Tunisia and Madagascar and the SWIO Islands or Trinidad & Tobago and Australia
417 clearly attests to the absence of a strict geographical structuring among the dataset of sequences.
418 Most interestingly, the fact that the phylogenetic tree retains at the global level the same topology
419 observed with *UMRVs* that infect rodents from Tunisia, supports the highly diffusing character of
420 these viruses and their capacity to infect a wide range of rodents, possibly through *R. rattus*. Viral
421 sequences from *C. gundi*, a species endemic to North Africa, clustered in two separate clades
422 with *UMRVs* sequences from *E. minor*, an endemic species from Madagascar. The fact that the
423 reservoir species harboring genetically related *UMRVs* are scattered in distant countries strongly
424 suggests the involvement of a third animal host which would act as a disseminating vector of

UMRVs. *Rattus sp.* is the ideal candidate to play this spreading role. Indeed, sequences from *C. gundi* and *E. minor* closely cluster with viral sequences detected in *R. rattus* from Tunisia and Madagascar. Several infection spillover events are actually detected that involve *R. rattus* as a partner i.e., *E. minor*/*R. rattus*, *C. gundi*/*R. rattus*, *M. natalensis*/*R. rattus*, *R. norvegicus*/*R. rattus*. Similar exchanges of *UMRVs* between *R. rattus* and other animal species belonging to other orders such as *Chiroptera* and *Afrosoricida* were previously described (14) which further stress the central role played by *Rattus sp.* in the transmission and the worldwide dissemination of these *UMRVs*. Our phylogeographic model supports this view. We consider that the *UMRVs*' distribution became a global phenomenon mainly as a consequence of the development of global trade (42) that spread infected rats over the five continents as previously described for other zoonoses (4, 5, 43). On the basis of this spatio-temporal reconstruction, we estimate that rodent associated *UMRVs* currently circulating worldwide, originated some 4000 years ago in Southern Africa. Some 500 years later, they split into two major viral lineages with a conservation of the ancestral one: clade B which gives origin to some African and SWIO Islands viruses around 2200 years ago and clade A1 which diverged to worldwide strains at around 3000 years before the present. *J* and *Mossman* viruses that were described in Australia, are closely-related to German and Zambian viruses, and were introduced between 1400 and 1500 years ago, respectively. Similarly *Nariva* virus from Trinidad & Tobago is closely-related to Zambian viruses and was introduced later. *Beilong* and *Tailam* viruses from China were related to SWIO Islands except Madagascar and were introduced about 1000 years ago. German viruses were introduced at about 2150 years ago. Noteworthy, the current circulation of *Rodentia* associated *UMRVs* from Tunisia is much more recent as it does not exceed 200 years ago.

Previous studies have revealed many migrations events in the *Murinea* subfamily between African regions and Asia during the last 12 million years (6, 44). Furthermore, dates of virus

449 introduction differ between the SWIO islands: the t_{MRCAs} indicates dates at around 2100 and 1200
450 years ago for Reunion Island and Madagascar, respectively; but only 150 years ago for
451 Seychelles and Mayotte. These dates are congruent with the evolutionary history of *Rattus*
452 introductions into the SWIO Islands (6, 45, 46), except maybe for Reunion Island. It has been
453 considered that *Rattus* introduction on Reunion began with the arrival of the first waves of
454 colonization on the island at around 1600 AD whereas our estimation would place the
455 introduction of *Rattus* much earlier. Although the latter estimate is plausible in light of oldest
456 description of the island by some travelers (45), it is more likely that this gap reflects a bias in our
457 data. In fact, this cluster is based solely on three sequences (two from *M. musculus*, whose
458 migratory history is likely identical to that of *Rattus* (46, 47); and a single sequence from *Rattus*
459 from Reunion Island), and consequently, without excluding a possibly poor estimation for that
460 particular introduction event. For Madagascar, our results are consistent with estimates from the
461 literature that places *Rattus* introduction at around 3000 before present (6, 46). For South Africa,
462 studies on *R. rattus* did not determine good estimates for the introduction of this species in this
463 country, but some authors believe that many introductions have occurred in this region, the oldest
464 was directly from India and/or the Middle East possibly with early Arab trading activities (6).
465 Outside we find a circulation of *UMRVs* in *R. pumilio* species which date back to around 250-500
466 years before present.

467 The possibility of different dates of virus introduction in the same location can be
468 explained by the occurrence of multiple introductions of the black rat into the zone as
469 demonstrated, for example, for *R. rattus* in Madagascar (46, 48). Likewise, the evolutionary
470 history of *R. rattus* described by Aplin et al. (6) suggests that commensalism arose multiple times
471 and in different geographic populations of black rats which is closely-related to the human
472 history as well as to the host-pathogen coevolution in this group of small mammals (42, 43). To

473 our knowledge this is the first observation ever described, where natural history and evolution
474 dynamics for a zoonotic virus, originating from wild fauna and hosted by wildlife species, could
475 have been impacted by commensalism related to human activities.

476 On the basis of phylogeographically reconstructed flow rates, *Rodentia-UMRVs* migrated
477 from Africa, worldwide. Interestingly, analysis of the population dynamics revealed that *UMRVs*
478 genetic diversity increased continuously until around 1000 years ago. Then, an exponential
479 increase in the viral diversity started at around the 10-11th century AD to reach a peak at around
480 the 16th century. In terms of genetic diversity, this picture could be explained first, by the
481 initiation and then deeper diversification, leading to a faster increase of the diverse
482 polymorphisms, necessary to create new viral lineages from Africa. The decline in the genetic
483 diversity that began on the 16th century and worsened over the last two hundred years until today
484 reflects a dramatic overall reduction of viral diversity. This phenomenon could have resulted
485 from several events: (i) an ancient drastic recombination event occurred in the past evolutionary
486 history; (ii) a founder effect with multiple host-jump transmissions, a geographical large-scale
487 expansion and global population bottleneck which purged preexisting genetic diversity,
488 selectively determined.

489 Regarding the first hypothesis, though we cannot exclude a remote infrequent recombination
490 event (49), it is difficult to detect such a phenomenon, as multiple viral generations have taken
491 place and purification selection can obscure the ancient age of viral lineages (50). Moreover, the
492 presence of some breakpoints could be attributed to heterotachy, an important process in protein
493 evolution (51). However, it is not the most suitable explanation, because if recombination can
494 shuffle mutations and purge deleterious variants, generating genotypes better fitting to the new
495 environment, yet, it still does not explain the persisting trend of the whole genetic diversity
496 contraction over a long period.

497 Regarding the founder effect hypothesis (small population size) it could be a possible
498 explanation, for both the increase, at different time/location points, of the viral diversification,
499 and the boost of genetic drift with mutations accumulation, leading to a faster loss of diversity
500 (52). Actually, the colonization by a few founders throughout periods of expansion, occurring at
501 multiple occasions, at large geographic scale, can create population bottlenecks and an iterative
502 loss of genetic diversity (53), and is in agreement with our data and the positive D Tajima value
503 estimate because of the shift in genetic composition owing to sampling effects. Hence, *UMRVs*'
504 global evolution would have been driven by a genetic drift compensated by a major purification
505 selection, which is a mode of selection outlined by its effect on frequency distributions of trait
506 values, and the slightly more exotic regimes of balancing selection (frequency-dependent
507 selection versus convergent selection) as a consequence of multiple constraints combinations.
508 Moreover, the weak evidence that some codons could be evolving under positive selection can
509 indicate adaptive fine-tuning, may be as a result of cross-species transmission (54).
510 Most interestingly the 11th century represented a dramatic turning point in human history of
511 Europe and the Mediterranean. This is the beginning of the Crusades and Wars with their large
512 population displacement and mixing, and the expansion on a large scale of the international trade
513 (55). Between the 10th and the 15th century, political and socioeconomic changes sped up the
514 opening up of regions, countries and continents: wars, large urban developments which were
515 flourishing (56) and changes in land use and cultivation methods towards intensive agriculture
516 (57). The recurrent bouts of bubonic Plague in which *Rattus* has played the role of the
517 disseminating vector through maritime trade, illustrate the health consequences of such global
518 revolution (43, 58). The history of black rat, a native of the Indian subcontinent, refers to one
519 type of lineage (lineage I of the complex *R. rattus*) that became one of the most widespread
520 species of mammals in the world (48). *Rattus* diffused worldwide accompanying human

521 displacements and maritime transport (59), including through the Oman Gulf and the Indian
522 Ocean, two important commercial routes between Asia and the Mediterranean via the Eastern
523 African coast (45, 60). In this context, the evolutionary history of the *Rodentia UMRVs* seems
524 particularly closely connected to the black rat history. Besides, this could be evidenced by the
525 fact that the number of lineages increased concomitantly with the decline in genetic diversity
526 observed among the PVs populations (Fig. 4b). Strikingly, in all the *UMRVs* studied here, our
527 estimates of the genetic diversity for the partial L-gene, are between 1000 and 5000 and, hence,
528 considerably higher than those previously recorded for the structural N and H genes in MeV (61)
529 and PPRV (62), reported by the respective authors themselves as being an unexpected very low
530 genetic diversity. However, our results are congruent with other viruses such as HIV (63) and
531 hepatitis C (64). From an epidemiological point of view, the increased number of lineages
532 probably reflects the fact that *Rattus* established epidemiological bridges between different
533 species. Since the 16th century, multiple exploratory expeditions from Europe to the rest of the
534 world (such those of Alfonso de Albuquerque, Vasco de Gama, Ferdinand Magellan, James Cook
535 (65), etc.) increased the dispersion of *Rattus*. In Madagascar, they coincided with an increasing
536 anthropogenic pressure causing major ecological disturbances, the destruction of primary forests
537 and habitat fragmentation (66) that have facilitated *Rattus* invasion, and the large expansion of
538 this alien species over endemic species (67). In Europe and Africa, there was an intensification of
539 agriculture and modern farming (68, 69). All these elements support the role of *Rattus* as a
540 pathogen spreader at the local, regional and global scales. Further studies are needed to evaluate
541 how other reservoirs hosts (particularly, *Chiroptera* and/or *Afrosoricida*), might have fueled the
542 *UMRVs* natural history. In term of migration pattern, they will likely bring little to the story as
543 most of the available sequences for these two orders are from species endemic to Madagascar
544 However, it may change the host origin of the ancestral viral lineage as it is not known which

among the *Chiroptera*, *Tenrecidae* or *Rodentia* stands as the ancestral reservoir of *UMRVs* in Africa, from which these zoonotic viruses have started their global diffusion. The specificity of interactions between these viruses, their potential vectors and reservoir hosts, the co-infection with other pathogens and the consequences of infections in humans and animals constitute a research priority to predict future emerging infections with epidemic and pandemic potential.

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Figure legends

Fig. 1. Maximum likelihood phylogeny of the partial L-gene of the *Paramyxoviridae* family including the Tunisian paramyxoviruses. PV sequences are distributed according to the geographic location of sampling sites and are colored according to the host species: *Psammomys obesus*, *Meriones shawi*, *Rattus rattus* and *Ctenodactylus gundi* colored in red, green, blue and purple; respectively. Values at node points indicate Bayesian posterior probabilities. Virus designations were as follow: Virus name or virus affiliation/typical host/accession number/origin/collection year/host Subfamily/host Family. Abbreviations used are detailed in Supplementary Table S1 in Supplementary material. Rabies virus (JQ595353) was used as an outgroup.

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Fig. 3. Maximum clade credibility tree of *Rodentia* associated *UMRVs*. The phylogenetic relationships and temporal evolutionary history have been estimated by molecular clock analysis. Branch lengths are temporally scaled, and the x-axis shows the time unit (years before present). The branches of the tree are colored on the basis of the most probable location of their descendent

nodes as specified in the figure. Geographical groups are identified according to the origin of the *UMRVs*. Viruses preceded by small Colored Square indicate a spillover event between *Rattus sp.* and other *Rodentia* species with the color indicating the location of this spillover. Values at node points indicate Bayesian posterior probabilities and the 95% HPD. Virus designations are as follows: Virus name or virus affiliation/typical host/accession number/origin/collection year/host Subfamily/host Family. Abbreviations used are detailed in Supplementary Table S2 in Supplementary material.

Fig. 4. Bayesian skygrid plots (BSP), inferred from partial L-gene, for *Rodentia* associated *UMRVs*. BSP depict viral population dynamics and the changing levels of genetic diversity (**a**) and the effective number of viral lineages (**b**) (y axis; log10 scale) over time (x axis; calendar years) for *Rodentia* associated *UMRVs* lineages, showing the median estimate (solid line) and credibility interval (blue area). The vertical dotted line represents the upper limit of the root height, with the mean t_{MRCAs} at the origin.

785 **Table 1.** Morphometric and demographic descriptions of rodents from Tunisia: *Psammomys* (*P.*)

786 *obesus*, *Meriones* (*M.*) *shawi*, *Rattus* (*R.*) *rattus* and *Ctenodactylus* (*C.*) *gundi.*

	P. obesus	***M. shawi***	***R. rattus***	***C. gundi***
Governorates (n (%))				
Sidi Bouzid	40 (100)	40 (100)	40 (100)	20 (50)
Tataouine	/	/	/	20 (50)
Sex (n (%))				
Male	23 (57.5)	15 (37.5)	22 (55)	20 (50)
Female	17 (42.5)	25 (62.5)	18 (45)	20 (50)
Standard morphometric parameters (Mean±SD)				
Weight (g)	124±24.9	81.83±26.34	98.13±33.67	224.53±54.85
Ear length (mm)	16.15±1.02	18.06±2.78	21.63±1.69	18.08±1.28
Head and body length (mm)	153.15±9.88	163.33±28.31	163.35±24.7	198.1±16.31
Tail length (mm)	128.28±7.81	119.09±64.37	181.43±60.91	28.75±9.87
Hind Feet length (mm)	36.18±1.33	36.23±4.17	33.08±2.55	41.93±2.26
Eyes lenses weight (Mean±SD)				
ELW (g)	39.21±6.77	66.19±20.73	52.05±18.41	92.50±32.57

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790 **Table 2.** Detection by RT-PCR of paramyxovirus infection in rodents from Tunisia according to
791 the sampling site and to the biometric parameters. *Psammomys* (*P.*) *obesus*, *Meriones* (*M.*) *shawi*,
792 *Rattus* (*R.*) *rattus* and *Ctenodactylus* (*C.*) *gundi*.

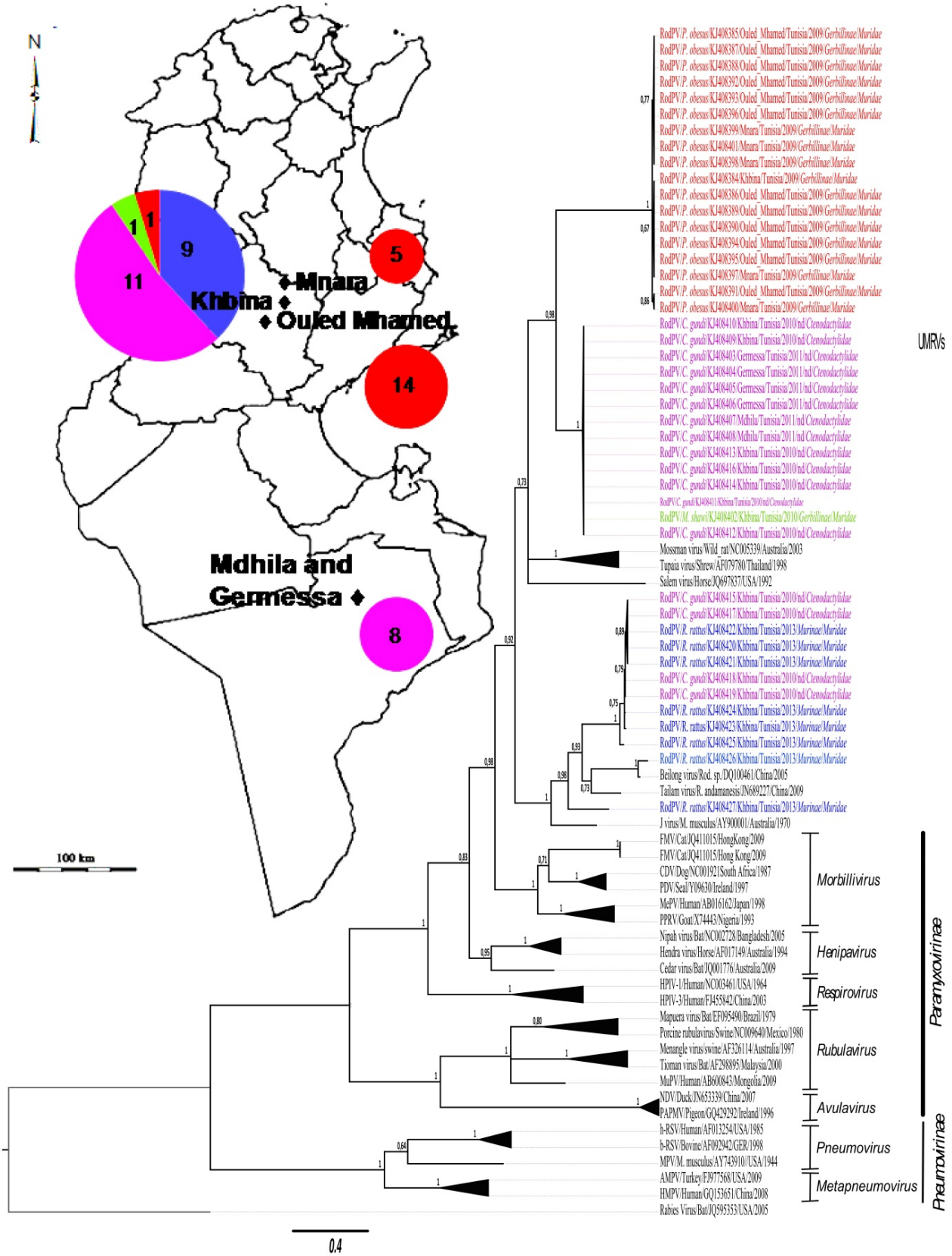
	P. obesus **(n = 40)**	***M. shawi*** **(n = 40)**	***R. rattus*** **(n = 40)**	***C. gundi*** **(n = 40)**
PVs infection (%, *P*)	20 (50, $< 10^{-3}$)	1 (2.5, $< 10^{-3}$)	9 (22.5, $< 10^{-3}$)	19 (47.5, $< 10^{-3}$)
According to the capture sites (n (%))				
Sidi Bouzid	20 (100)	1 (100)	9 (100)	11 (57.9)
Tataouine	/	/	/	8 (42.1)
P	/	/	/	NS
According to gender (n (%))				
Male	14 (70)	1 (100)	6 (66.7)	8 (42.1)
Female	6 (30)	/	3 (33.3)	11 (57.9)
P	NS	/	NS	NS
According to the standard morphometric parameters (Mean±SD (*P*))				
Weight (g)	129±70 (NS)	35	96.67±28.28 (NS)	227.95±46.30 (NS)
Ear length (mm)	16.35±1.13 (NS)	14.00	21.67±0.86 (NS)	18.21±1.22 (NS)
Head and body length (mm)	155.30±10.09 (NS)	105.00	164.89±16.72 (NS)	199.11±11.65 (NS)
Tail length (mm)	125.95±7.30 (NS)	152.00	199.11±22.72 (NS)	28.16±9.48 (NS)
Hind Feet length (mm)	36.00±1.37 (NS)	27.00	33.44±1.59 (NS)	42.21±1.58 (NS)
According to the eyes lenses weight (Mean±SD (*P*))				
ELW (g)	41.21±4.39 (NS)	40	47.70±21.31 (NS)	93.86±27.76 (NS)

793 NS: Not Significant.
794
795

796 **Table 3.** Summary of selection pressures acting in *Rodentia UMRVs* versus Morbilliviruses.

Genus	Codon-based nucleic acid gene level											Protein gene level			
	N° of seq (Partial L-gene)	D Tajima Value	SLAC[a] Positively/Negatively selected sites		FEL[a] Positively/Negatively selected sites		REL[a] Positively/Negatively selected sites		MEME[a] (codon position)	FUBAR[b]	Integrative Selection (at least one method)	BGM[c] (codon position)	N° of seq (Partial L-gene)	DEPS (N° residues)	FADE
			Pos.	Neg.	Pos.	Neg.	Pos.	Neg.							
Rodent *UMRVs*	120	+4.2839	None	131	None	138	NA	NA	3 (31, 62, 85)	142	146	3 (30, 84, 94)	80	62 (19)	72
PPRV	15	+0.0925	None	12	None	24	None	71	None	22	71	None	6	None	4
MeV	17	-1.0352	None	6	None	11	None	None	None	7	11	None	10	None	4
CDV	11	-0.4934	None	6	None	19	None	None	None	14	20	None	6	None	1
FMV	6	-0.2620	None	1 (109)	None	28	None	None	None	12	28	None	5	None	None

797 [a] $p < 0.1$ (no positively selected sites were detected, except for one site (62) with MEME at $p < 0.05$). [b] Posterior prob > 0.90. [c] Posterior prob >
798 0.95. seq: sequences.
799 For nucleic acid level, we used the HKY85 model, except for Rod-*UMRVs* (GTR) with input neighbor-joining trees. For protein gene level, we
800 used the JTT model. Directional Evolution Protein Sequences (DEPS) in *UMRVs* (selective sweeps): 23 residues substitutions evolving under a
801 Frequency-dependent selection, 73 residues substitutions evolving under a Convergent evolution and 18 sites evolving under a Balancing selection
802 (Frequency-dependent selection versus Convergent evolution). Except for the D Tajima statistical test (Mega6), all the analyses have been
803 performed via the Datamonkey facility.
804



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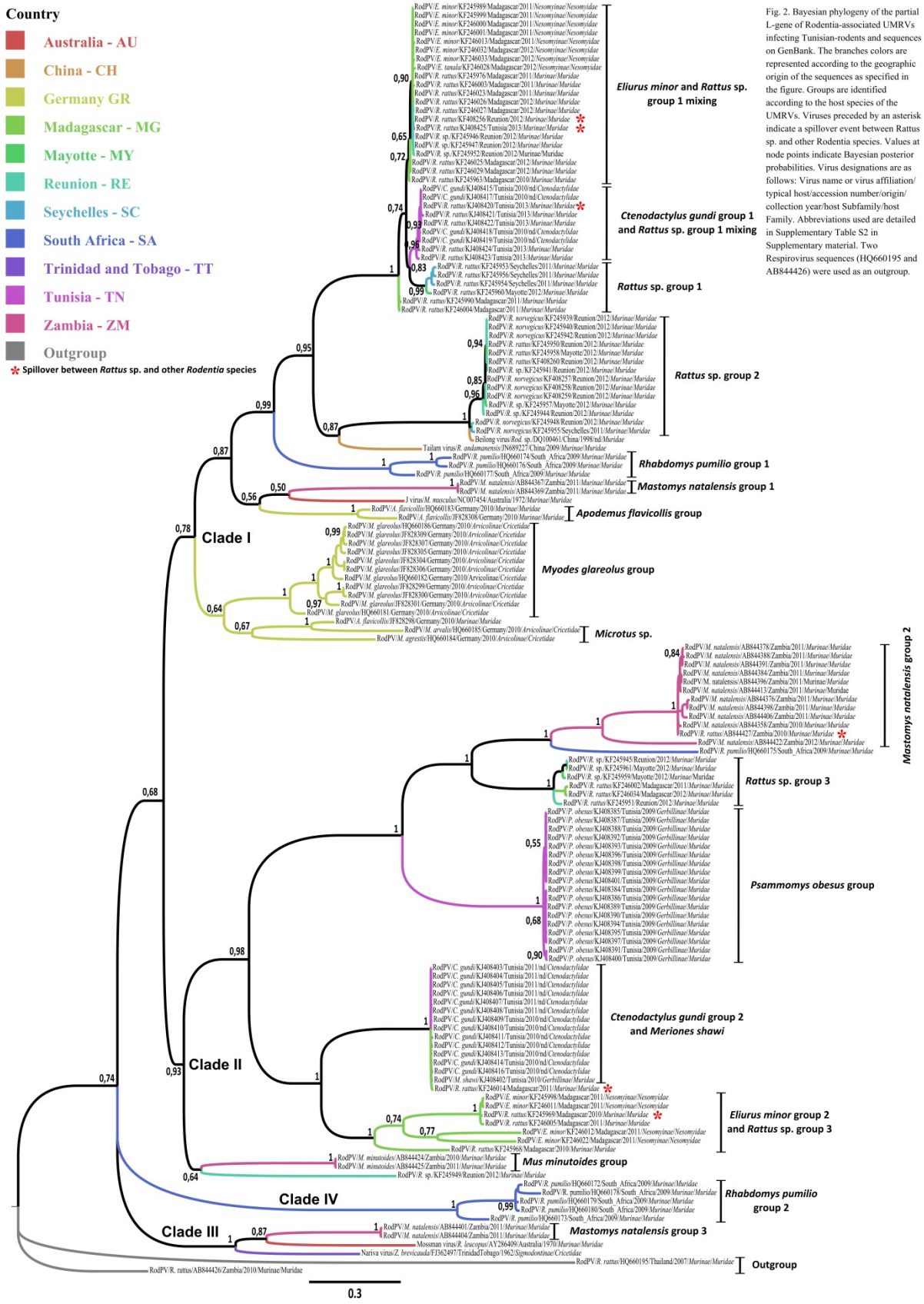

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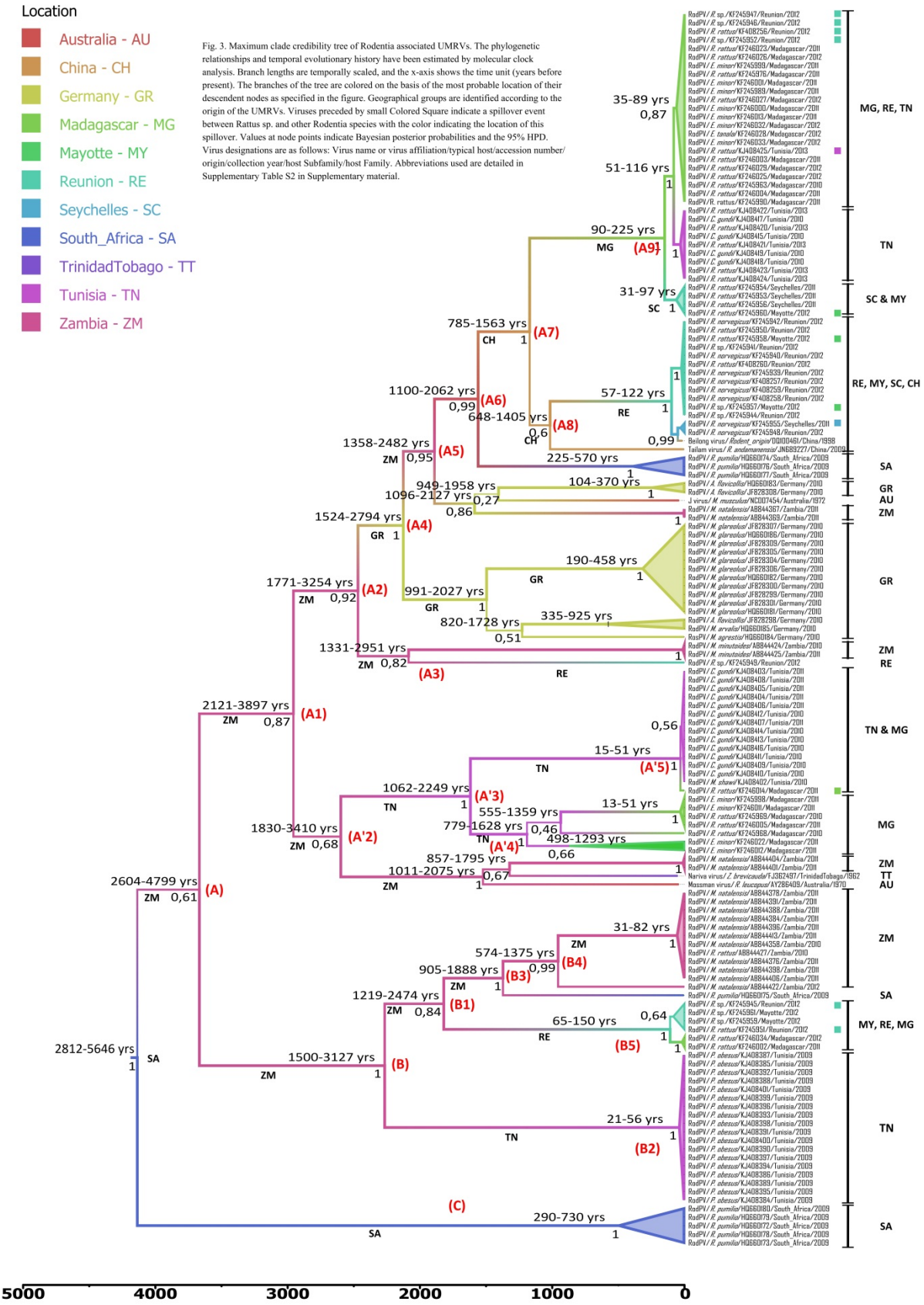

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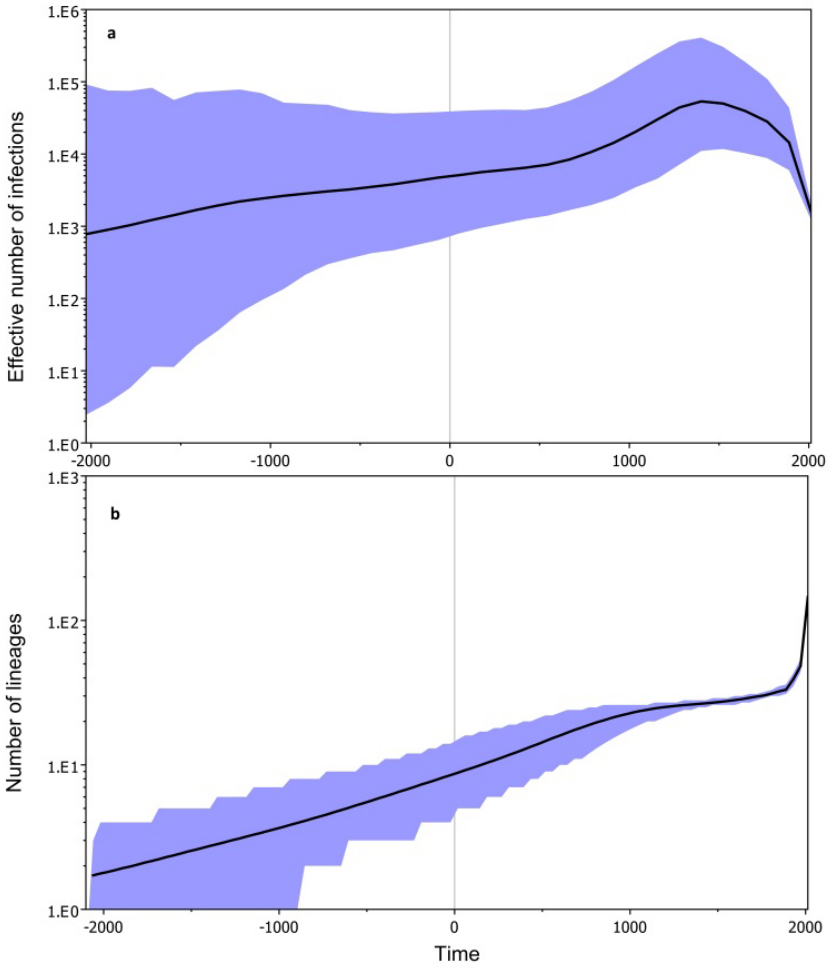

Supplementary Material:

Supplementary Figure and Table Legends:

Supplementary Figure S1: The hypothetical worldwide dispersion patterns of *Rodentia* associated *UMRV* isolates as reconstructed on the basis of significant genetic flow rates and dated phylogeny. Only the rates supported by a Bayes Factor (BF) greater than 3 are shown. The map was reconstructed using SPREAD.

Supplementary Table S1: Viruses used for the classification of RodPVs-Tun among *Paramyxoviridae* family in Figure 1.

Supplementary Table S2: Viruses used for the phylogenetic and phylogeographic analysis of *Rodentia* associated *UMRV* in Figures 2 and 3.

Supplementary Table S3: Viruses used for the estimate of the evolutionary rate change for the partial L-gene among the Measles virus.

Supplementary Table S4: Significant diffusion rates between countries at Bayesian phylogeographic analysis. Posterior probability of the rates k (pk) and Bayes factor (BF) of the significant migration rates as revealed by the Skygrid symmetric model.

Supplementary Text 1: Estimate of the evolutionary rate change for the partial L-gene among the Measles virus.

As *Rodentia* PVs presented in this study are very close Morbilli-related viruses (1-3) and for some authors probably could be considered as sister genera (4) we used the Measles virus (MeV) as a reference for the analysis of evolutionary changes of *UMRVs*. Out of 189 complete genomes, 24 MeV partial L-gene sequences globally distributed have been selected according to the criterion of the date of collection availability, spanning the years between 1989 and 2014 (Supplementary Table S3). Besides, one might think that only 24 sequences of the MeV may lead to inaccurate estimates; however, to compensate this aspect, the L-gene may provide the most reliable evolutionary signal at the family level. The best-fit substitution model was K2 + G, but we used HKY85 + G the nearest close relative best-fit model available in Beauti (Beast package 1.8.2). We used serial samples of MeV for the partial L-gene to estimate the rate of nucleotide substitution and the time to the most recent common ancestor (t_{MRCAs}), using the Bayesian MCMC method with a coalescent skyline plot method, allowing the analysis of the distribution branch length among collected viruses at different times sampled from millions of trees. We performed a lognormal relaxed molecular clock (5) and a random local clock (RLC) (6), either with a constant population size or a skyline demographic model. The RLC model submits and analyses a succession of different local molecular clocks, each potentially occurring on any branch and extending over a contiguous part of the phylogeny. Runs were carried out with chain lengths of 100-200 millions. The output from Beast was analyzed using the program Tracer 1.6 (http://beast.bio.ed.ac.uk/Tracer). Obtained mean rate was used to the subsequent *UMRVs* phylogeographic analysis.

The majority of the set comprising the 145 sequences of *UMRVs* felt in a too small time interval, introducing too much bias to determine empirically the evolutionary history and

49 migration patterns of these viruses directly from our data set. This is due to the fact that
50 different field campaigns to collect samples have all been conducted between 2009 and 2013.
51 However, it is reasonable to assume that determining the evolutionary rate change a virus-
52 related of the same genera or from a virus that proceeds to the same common ancestor could
53 remain a good alternative and a reliable approximation in our case (L-gene). Parameter
54 estimates were consistent among models and similar values were obtained for all the analyses.
55 The evolutionary rate of the current circulating MeV for the L-gene was estimated to be 3.48
56 x 10^{-4} substitutions per site per year (95% HPD 3.94 x 10^{-5}, 6.74 x 10^{-4}), and coalescent
57 estimates place its recent emergence at around 1900 (~116 years from 2014, 95% HPD 30.46
58 to 245.53). The mean evolutionary rate and the t_{MRCAs} derived from the partial L-gene were
59 consistent and quietly similar to those already described for other various genes of the current
60 circulating MeV. However, our values were slightly lower than those obtained for the
61 structural H and N genes (7, 8), but it was not unexpected, being considered that the
62 polymerase gene is the most conserved gene among the *Paramyxoviridae* to maintain crucial
63 structural domains such as multiple functions related to different replicative mechanisms (4,
64 9). This molecular clock has been used for the phylogeographic analysis.
65
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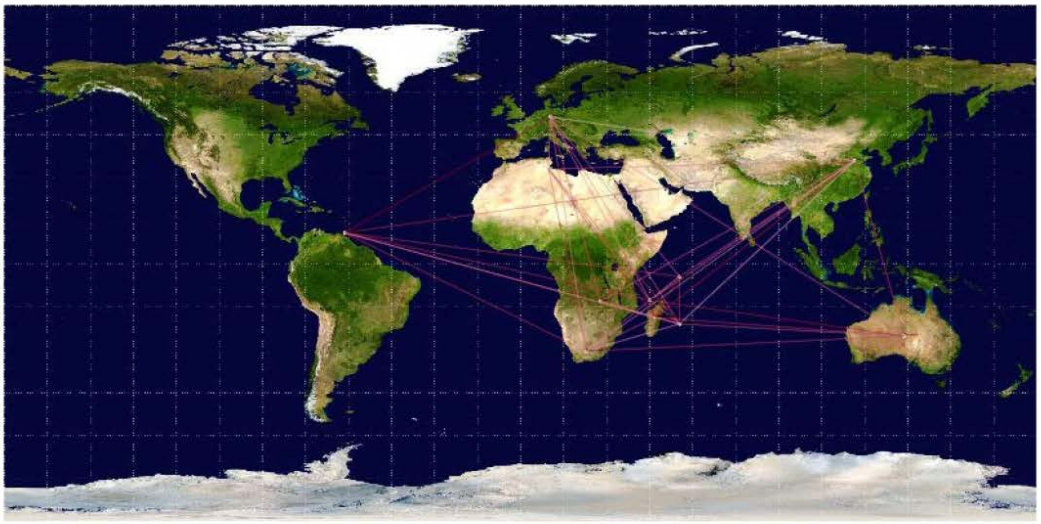

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Supplementary Table S1: Viruses used for the classification of RodPVs-Tun among *Paramyxoviridae* family in Figure 1.

Virus ID [a,b]	Accession numbers	Country	Collection year	Host
RodPV/C. gundi/KJ408415/Tunisia/2010/nd/Ctenodactylidae	KJ408415	Tunisia	2010	*Ctenodactylus gundi*
RodPV/C. gundi/KJ408417/Tunisia/2010/nd/Ctenodactylidae	KJ408417	Tunisia	2010	*Ctenodactylus gundi*
RodPV/C. gundi/KJ408418/Tunisia/2010/nd/Ctenodactylidae	KJ408418	Tunisia	2010	*Ctenodactylus gundi*
RodPV/C. gundi/KJ408419/Tunisia/2010/nd/Ctenodactylidae	KJ408419	Tunisia	2010	*Ctenodactylus gundi*
RodPV/R. rattus/KJ408424/Tunisia/2013/Murinae/Muridae	KJ408424	Tunisia	2013	*Rattus rattus*
RodPV/R. rattus/KJ408422/Tunisia/2013/Murinae/Muridae	KJ408422	Tunisia	2013	*Rattus rattus*
RodPV/R. rattus/KJ408423/Tunisia/2013/Murinae/Muridae	KJ408423	Tunisia	2013	*Rattus rattus*
RodPV/R. rattus/KJ408420/Tunisia/2013/Murinae/Muridae	KJ408420	Tunisia	2013	*Rattus rattus*
RodPV/R. rattus/KJ408421/Tunisia/2013/Murinae/Muridae	KJ408421	Tunisia	2013	*Rattus rattus*
RodPV/R. rattus/KJ408425/Tunisia/2013/Murinae/Muridae	KJ408425	Tunisia	2013	*Rattus rattus*
Tailam virus/R. andamanensis/JN689227/China/2009	JN689227	China	2009	*Rattus andamanensis*
RodPV/R. rattus/KJ408426/Tunisia/2013/Murinae/Muridae	KJ408426	Tunisia	2013	*Rattus rattus*
Beilong virus/Rod. sp./DQ100461/China/2005	DQ100461	China	2005	*Rodent* sp.
RodPV/R. rattus/KJ408427/Tunisia/2013/Murinae/Muridae	KJ408427	Tunisia	2013	*Rattus rattus*
J virus/M. musculus/AY900001/Australia/1970	AY900001	Australia	1970	*Mus musculus*
RodPV/C. gundi/KJ408410/Tunisia/2010/nd/Ctenodactylidae	KJ408410	Tunisia	2010	*Ctenodactylus gundi*
RodPV/C. gundi/KJ408409/Tunisia/2010/nd/Ctenodactylidae	KJ408409	Tunisia	2010	*Ctenodactylus gundi*
RodPV/C. gundi/KJ408403/Tunisia/2011/nd/Ctenodactylidae	KJ408403	Tunisia	2011	*Ctenodactylus gundi*
RodPV/C. gundi/KJ408404/Tunisia/2011/nd/Ctenodactylidae	KJ408404	Tunisia	2011	*Ctenodactylus gundi*
RodPV/C. gundi/KJ408405/Tunisia/2011/nd/Ctenodactylidae	KJ408405	Tunisia	2011	*Ctenodactylus gundi*
RodPV/C. gundi/KJ408406/Tunisia/2011/nd/Ctenodactylidae	KJ408406	Tunisia	2011	*Ctenodactylus gundi*
RodPV/C. gundi/KJ408407/Tunisia/2011/nd/Ctenodactylidae	KJ408407	Tunisia	2011	*Ctenodactylus gundi*
RodPV/C. gundi/KJ408408/Tunisia/2011/nd/Ctenodactylidae	KJ408408	Tunisia	2011	*Ctenodactylus gundi*
RodPV/C. gundi/KJ408413/Tunisia/2010/nd/Ctenodactylidae	KJ408413	Tunisia	2010	*Ctenodactylus gundi*
RodPV/C. gundi/KJ408416/Tunisia/2010/nd/Ctenodactylidae	KJ408416	Tunisia	2010	*Ctenodactylus gundi*
RodPV/C. gundi/KJ408414/Tunisia/2010/nd/Ctenodactylidae	KJ408414	Tunisia	2010	*Ctenodactylus gundi*
RodPV/C. gundi/KJ408411/Tunisia/2010/nd/Ctenodactylidae	KJ408411	Tunisia	2010	*Ctenodactylus gundi*
RodPV/M. shawi/KJ408402/Tunisia/2010/Gerbillinae/Muridae	KJ408402	Tunisia	2010	*Meriones shawi*
RodPV/C. gundi/KJ408412/Tunisia/2010/nd/Ctenodactylidae	KJ408412	Tunisia	2010	*Ctenodactylus gundi*
Mossman virus/Wild_rat/NC005339/Australia/2003	NC005339	Australia	2003	*Wild rat*
Tupaia virus/Shrew/AF079780/Thailand/1998	AF079780	Thailand	1998	*Shrew*

FMV/Cat/JQ411015/HongKong/2009	JQ411015	Hong Kong	2009	*Felis catus*
FMV/Cat/JQ411015/HongKong/2009	JQ411015	Hong Kong	2009	*Felis catus*
MePV/Human/AB016162/Japan/1998	AB016162	Japan	1998	*Human*
PPRV/Goat/X74443/Nigeria/1993	X74443	Nigeria	1993	*Goat*
CDV/Dog/NC001921South Africa/1987	NC001921	South Africa	1987	*Dog*
PDV/Seal/Y09630/Ireland/1997	Y09630	Ireland	1997	*Seal*
RodPV/P. obesus/KJ408384/Tunisia/2009/Gerbillinae/Muridae	KJ408384	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408385/Tunisia/2009/Gerbillinae/Muridae	KJ408385	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408386/Tunisia/2009/Gerbillinae/Muridae	KJ408386	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408387/Tunisia/2009/Gerbillinae/Muridae	KJ408387	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408388/Tunisia/2009/Gerbillinae/Muridae	KJ408388	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408389/Tunisia/2009/Gerbillinae/Muridae	KJ408389	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408390/Tunisia/2009/Gerbillinae/Muridae	KJ408390	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408391/Tunisia/2009/Gerbillinae/Muridae	KJ408391	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408392/Tunisia/2009/Gerbillinae/Muridae	KJ408392	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408393/Tunisia/2009/Gerbillinae/Muridae	KJ408393	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408394/Tunisia/2009/Gerbillinae/Muridae	KJ408394	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408396/Tunisia/2009/Gerbillinae/Muridae	KJ408396	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408395/Tunisia/2009/Gerbillinae/Muridae	KJ408395	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408397/Tunisia/2009/Gerbillinae/Muridae	KJ408397	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408399/Tunisia/2009/Gerbillinae/Muridae	KJ408399	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408401/Tunisia/2009/Gerbillinae/Muridae	KJ408401	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408400/Tunisia/2009/Gerbillinae/Muridae	KJ408400	Tunisia	2009	*Psammomys obesus*
RodPV/P. obesus/KJ408398/Tunisia/2009/Gerbillinae/Muridae	KJ408398	Tunisia	2009	*Psammomys obesus*
Nipah virus/Bat/NC002728/Bangladesh/2005	NC002728	Bangladesh	2005	Bat
Hendra virus/Horse/AF017149/Australia/1994	AF017149	Australia	1994	Horse
Cedar virus/Bat/JQ001776/Australia/2009	JQ001776	Australia	2009	Bat
Salem virus/Horse/JQ697837/USA/1992	JQ697837	USA	1992	Horse
HPIV-1/Human/NC003461/USA/1964	NC003461	USA	1964	Human
HPIV-3/Human/FJ455842/China/2003	FJ455842	China	2003	Human
Mapuera virus/Bat/EF095490/Brazil/1979	EF095490	Brazil	1979	Bat
Porcine rubulavirus/Swine/NC009640/Mexico/1980	NC009640	Mexico	1980	Swine
MuPV/Human/AB600843/Mongolia/2009	AB600843	Mongolia	2009	Human
Menangle virus/Swine/AF326114/Australia/1997	AF326114	Australia	1997	*Swine*
Tioman virus/Bat/AF298895/Malaysia/2000	AF298895	Malaysia	2000	*Bat*
NDV/Duck/JN653339/China/2007	JN653339	China	2007	*Duck*
PAPMV/Pigeon/GQ429292/Ireland/1996	GQ429292	Ireland	1996	*Pigeon*

h-RSV/Human/AF013254/USA/1985	AF013254	USA	1985	*Human*
b-RSV/Bovine/AF092942/GER/1998	AF092942	Germany	1998	*Bovine*
AMPV/Turkey/FJ977568/USA/2009	FJ977568	USA	2009	*Turkey*
HMPV/Human/GQ153651/China/2008	GQ153651	China	2008	*Human*
MPV/M. musculus/AY743910/USA/1944	AY743910	USA	1944	*Mus musculus*
Rabies Virus/Bat/JQ595353/USA/2005*	JQ595353	USA	2005	*Bat*

[a]: Virus name or virus affiliation/typical host/ accession number/origin/collection year/host Subfamily/host Family

[b]: AMPV = Avian Metapneumovirus; b-RSV = Bovine Respiratory Syncitical Virus; C. gundi = *Ctenodactylus gundi*; CDV = Canine Distemper Virus; FMV = Feline Morbillivirus; HMPV = Human Metapneumovirus; HPIV = Human Parainfluenza Virus; h-RSV = Human Respiratory Syncitical Virus; M. musculus = *Mus musculus*; M. shawi = *Meriones shawi*; MePV = Measles Virus; MPV = Murine Pneumonia Virus; MuPV = Mumps Virus; nd = not defined; NDV = Newcastle Disease Virus; P. obesus = *Psammomys obesus*; PAPMV = Pigeon Paramyxovirus; PDV= Phocine Distemper Virus; PPRV = Peste des Petits Ruminents Virus; R. rattus= *Rattus rattus*; Rod. = Rodents; RodPV = Rodents Paramyxovirus; sp. = species.

*: Sequence of rabies virus used as outgroup.

Supplementary Table S2: Viruses used for the phylogenetic and phylogeographic analysis of *Rodentia* associated *UMRV* in Figures 2 and 3.

Virus ID [a,b]	Accession numbers	Country	Collection year	Host [b]
Beilong virus/Rodent_origin/DQ100461/China/1998/nd/Muridae	DQ100461	China	1998	*Rodent origin*
J virus/Mus_musculus/NC007454/Australia/1972/Murinae/Muridae	NC007454	Australia	1972	*Mus musculus*
Mossman virus/Rattus_leucopus/AY286409/Australia/1970/Murinae/Muridae	AY286409	Australia	1970	*Rattus_leucopus*
Nariva virus/Zygodontomys_brevicauda/FJ362497/TrinidadTobago/1962/ Sigmodontinae/Cricetidae	FJ362497	Trinidad Tobago	1962	*Zygodontomys brevicauda*
RodPV/Apodemus_flavicollis/HQ660183/Germany/2010/Murinae/Muridae	HQ660183	Germany	2010	*Apodemus_flavicollis*
RodPV/Apodemus_flavicollis/JF828298/Germany/2010/Murinae/Muridae	JF828298	Germany	2010	*Apodemus_flavicollis*
RodPV/Apodemus_flavicollis/JF828308/Germany/2010/Murinae/Muridae	JF828308	Germany	2010	*Apodemus_flavicollis*
RodPV/Ctenodactylus_gundi/KJ408403/Tunisia/2011/nd/Ctenodactylidae	KJ408403	Tunisia	2011	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408404/Tunisia/2011/nd/Ctenodactylidae	KJ408404	Tunisia	2011	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408405/Tunisia/2011/nd/Ctenodactylidae	KJ408405	Tunisia	2011	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408406/Tunisia/2011/nd/Ctenodactylidae	KJ408406	Tunisia	2011	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408407/Tunisia/2011/nd/Ctenodactylidae	KJ408407	Tunisia	2011	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408408/Tunisia/2011/nd/Ctenodactylidae	KJ408408	Tunisia	2011	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408409/Tunisia/2010/nd/Ctenodactylidae	KJ408409	Tunisia	2010	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408410/Tunisia/2010/nd/Ctenodactylidae	KJ408410	Tunisia	2010	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408411/Tunisia/2010/nd/Ctenodactylidae	KJ408411	Tunisia	2010	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408412/Tunisia/2010/nd/Ctenodactylidae	KJ408412	Tunisia	2010	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408413/Tunisia/2010/nd/Ctenodactylidae	KJ408413	Tunisia	2010	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408414/Tunisia/2010/nd/Ctenodactylidae	KJ408414	Tunisia	2010	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408415/Tunisia/2010/nd/Ctenodactylidae	KJ408415	Tunisia	2010	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408416/Tunisia/2010/nd/Ctenodactylidae	KJ408416	Tunisia	2010	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408417/Tunisia/2010/nd/Ctenodactylidae	KJ408417	Tunisia	2010	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408418/Tunisia/2010/nd/Ctenodactylidae	KJ408418	Tunisia	2010	*Ctenodactylus gundi*
RodPV/Ctenodactylus_gundi/KJ408419/Tunisia/2010/nd/Ctenodactylidae	KJ408419	Tunisia	2010	*Ctenodactylus gundi*
RodPV/Eliurus_minor/KF245989/Madagascar/2011/Nesomyinae/Nesomyidae	KF245989	Madagascar	2011	*Eliurus minor*
RodPV/Eliurus_minor/KF245998/Madagascar/2011/Nesomyinae/Nesomyidae	KF245998	Madagascar	2011	*Eliurus minor*
RodPV/Eliurus_minor/KF245999/Madagascar/2011/Nesomyinae/Nesomyidae	KF245999	Madagascar	2011	*Eliurus minor*
RodPV/Eliurus_minor/KF246000/Madagascar/2011/Nesomyinae/Nesomyidae	KF246000	Madagascar	2011	*Eliurus minor*
RodPV/Eliurus_minor/KF246001/Madagascar/2011/Nesomyinae/Nesomyidae	KF246001	Madagascar	2011	*Eliurus minor*
RodPV/Eliurus_minor/KF246011/Madagascar/2011/Nesomyinae/Nesomyidae	KF246011	Madagascar	2011	*Eliurus minor*

RodPV/Eliurus_minor/KF246012/Madagascar/2011/Nesomyinae/Nesomyidae	KF246012	Madagascar	2011	*Eliurus minor*
RodPV/Eliurus_minor/KF246013/Madagascar/2011/Nesomyinae/Nesomyidae	KF246013	Madagascar	2011	*Eliurus minor*
RodPV/Eliurus_minor/KF246022/Madagascar/2011/Nesomyinae/Nesomyidae	KF246022	Madagascar	2011	*Eliurus minor*
RodPV/Eliurus_minor/KF246032/Madagascar/2012/Nesomyinae/Nesomyidae	KF246032	Madagascar	2012	*Eliurus minor*
RodPV/Eliurus_minor/KF246033/Madagascar/2012/Nesomyinae/Nesomyidae	KF246033	Madagascar	2012	*Eliurus minor*
RodPV/Eliurus_tanala/KF246028/Madagascar/2012/Nesomyinae/Nesomyidae	KF246028	Madagascar	2012	*Eliurus tanala*
RodPV/Mastomys_natalensis/AB844358/Zambia/2010/Murinae/Muridae	AB844358	Zambia	2010	*Mastomys natalensis*
RodPV/Mastomys_natalensis/AB844367/Zambia/2011/Murinae/Muridae	AB844367	Zambia	2011	*Mastomys natalensis*
RodPV/Mastomys_natalensis/AB844369/Zambia/2011/Murinae/Muridae	AB844369	Zambia	2011	*Mastomys natalensis*
RodPV/Mastomys_natalensis/AB844376/Zambia/2011/Murinae/Muridae	AB844376	Zambia	2011	*Mastomys natalensis*
RodPV/Mastomys_natalensis/AB844378/Zambia/2011/Murinae/Muridae	AB844378	Zambia	2011	*Mastomys natalensis*
RodPV/Mastomys_natalensis/AB844384/Zambia/2011/Murinae/Muridae	AB844384	Zambia	2011	*Mastomys natalensis*
RodPV/Mastomys_natalensis/AB844388/Zambia/2011/Murinae/Muridae	AB844388	Zambia	2011	*Mastomys natalensis*
RodPV/Mastomys_natalensis/AB844391/Zambia/2011/Murinae/Muridae	AB844391	Zambia	2011	*Mastomys natalensis*
RodPV/Mastomys_natalensis/AB844396/Zambia/2011/Murinae/Muridae	AB844396	Zambia	2011	*Mastomys natalensis*
RodPV/Mastomys_natalensis/AB844398/Zambia/2011/Murinae/Muridae	AB844398	Zambia	2011	*Mastomys natalensis*
RodPV/Mastomys_natalensis/AB844401/Zambia/2011/Murinae/Muridae	AB844401	Zambia	2011	*Mastomys natalensis*
RodPV/Mastomys_natalensis/AB844404/Zambia/2011/Murinae/Muridae	AB844404	Zambia	2011	*Mastomys natalensis*
RodPV/Mastomys_natalensis/AB844406/Zambia/2011/Murinae/Muridae	AB844406	Zambia	2011	*Mastomys natalensis*
RodPV/Mastomys_natalensis/AB844413/Zambia/2011/Murinae/Muridae	AB844413	Zambia	2011	*Mastomys natalensis*
RodPV/Mastomys_natalensis/AB844422/Zambia/2012/Murinae/Muridae	AB844422	Zambia	2012	*Mastomys natalensis*
RodPV/Meriones_shawi/KJ408402/Tunisia/2010/Gerbillinae/Muridae	KJ408402	Tunisia	2010	*Meriones_shawi*
RodPV/Microtus_arvalis/HQ660185/Germany/2010/Arvicolinae/Cricetidae	HQ660185	Germany	2010	*Microtus arvalis*
RodPV/Mus_minutoides/AB844424/Zambia/2010/Murinae/Muridae	AB844424	Zambia	2010	*Mus minutoides*
RodPV/Mus_minutoides/AB844425/Zambia/2011/Murinae/Muridae	AB844425	Zambia	2011	*Mus minutoides*
RodPV/Myodes_glareolus/HQ660181/Germany/2010/Arvicolinae/Cricetidae	HQ660181	Germany	2010	*Myodes glareolus*
RodPV/Myodes_glareolus/HQ660182/Germany/2010/Arvicolinae/Cricetidae	HQ660182	Germany	2010	*Myodes glareolus*
RodPV/Myodes_glareolus/HQ660186/Germany/2010/Arvicolinae/Cricetidae	HQ660186	Germany	2010	*Myodes glareolus*
RodPV/Myodes_glareolus/JF828299/Germany/2010/Arvicolinae/Cricetidae	JF828299	Germany	2010	*Myodes glareolus*
RodPV/Myodes_glareolus/JF828300/Germany/2010/Arvicolinae/Cricetidae	JF828300	Germany	2010	*Myodes glareolus*
RodPV/Myodes_glareolus/JF828301/Germany/2010/Arvicolinae/Cricetidae	JF828301	Germany	2010	*Myodes glareolus*
RodPV/Myodes_glareolus/JF828304/Germany/2010/Arvicolinae/Cricetidae	JF828304	Germany	2010	*Myodes glareolus*
RodPV/Myodes_glareolus/JF828305/Germany/2010/Arvicolinae/Cricetidae	JF828305	Germany	2010	*Myodes glareolus*
RodPV/Myodes_glareolus/JF828306/Germany/2010/Arvicolinae/Cricetidae	JF828306	Germany	2010	*Myodes glareolus*
RodPV/Myodes_glareolus/JF828307/Germany/2010/Arvicolinae/Cricetidae	JF828307	Germany	2010	*Myodes glareolus*
RodPV/Myodes_glareolus/JF828309/Germany/2010/Arvicolinae/Cricetidae	JF828309	Germany	2010	*Myodes glareolus*
RodPV/Psammomys_obesus/KJ408384/Tunisia/2009/Gerbillinae/Muridae	KJ408384	Tunisia	2009	*Psammomys obesus*

RodPV/Psammomys_obesus/KJ408385/Tunisia/2009/Gerbillinae/Muridae	KJ408385	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408386/Tunisia/2009/Gerbillinae/Muridae	KJ408386	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408387/Tunisia/2009/Gerbillinae/Muridae	KJ408387	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408388/Tunisia/2009/Gerbillinae/Muridae	KJ408388	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408389/Tunisia/2009/Gerbillinae/Muridae	KJ408389	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408390/Tunisia/2009/Gerbillinae/Muridae	KJ408390	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408391/Tunisia/2009/Gerbillinae/Muridae	KJ408391	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408392/Tunisia/2009/Gerbillinae/Muridae	KJ408392	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408393/Tunisia/2009/Gerbillinae/Muridae	KJ408393	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408394/Tunisia/2009/Gerbillinae/Muridae	KJ408394	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408395/Tunisia/2009/Gerbillinae/Muridae	KJ408395	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408396/Tunisia/2009/Gerbillinae/Muridae	KJ408396	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408397/Tunisia/2009/Gerbillinae/Muridae	KJ408397	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408398/Tunisia/2009/Gerbillinae/Muridae	KJ408398	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408399/Tunisia/2009/Gerbillinae/Muridae	KJ408399	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408400/Tunisia/2009/Gerbillinae/Muridae	KJ408400	Tunisia	2009	*Psammomys obesus*
RodPV/Psammomys_obesus/KJ408401/Tunisia/2009/Gerbillinae/Muridae	KJ408401	Tunisia	2009	*Psammomys obesus*
RodPV/Rattus_norvegicus/KF245939/Reunion/2012/Murinae/Muridae	KF245939	Reunion	2012	*Rattus norvegicus*
RodPV/Rattus_norvegicus/KF245940/Reunion/2012/Murinae/Muridae	KF245940	Reunion	2012	*Rattus norvegicus*
RodPV/Rattus_norvegicus/KF245942/Reunion/2012/Murinae/Muridae	KF245942	Reunion	2012	*Rattus norvegicus*
RodPV/Rattus_norvegicus/KF245948/Reunion/2012/Murinae/Muridae	KF245948	Reunion	2012	*Rattus norvegicus*
RodPV/Rattus_norvegicus/KF245955/Seychelles/2011/Murinae/Muridae	KF245955	Seychelles	2011	*Rattus norvegicus*
RodPV/Rattus_norvegicus/KF408257/Reunion/2012/Murinae/Muridae	KF408257	Reunion	2012	*Rattus norvegicus*
RodPV/Rattus_norvegicus/KF408258/Reunion/2012/Murinae/Muridae	KF408258	Reunion	2012	*Rattus norvegicus*
RodPV/Rattus_norvegicus/KF408259/Reunion/2012/Murinae/Muridae	KF408259	Reunion	2012	*Rattus norvegicus*
RodPV/Rattus_rattus/HQ660195/Thailand/2007/Murinae/Muridae*	HQ660195	Thailand	2007	*Rattus rattus*
RodPV/Rattus_rattus/KF245950/Reunion/2012/Murinae/Muridae	KF245950	Reunion	2012	*Rattus rattus*
RodPV/Rattus_rattus/KF245951/Reunion/2012/Murinae/Muridae	KF245951	Reunion	2012	*Rattus rattus*
RodPV/Rattus_rattus/KF245953/Seychelles/2011/Murinae/Muridae	KF245953	Seychelles	2011	*Rattus rattus*
RodPV/Rattus_rattus/KF245954/Seychelles/2011/Murinae/Muridae	KF245954	Seychelles	2011	*Rattus rattus*
RodPV/Rattus_rattus/KF245956/Seychelles/2011/Murinae/Muridae	KF245956	Seychelles	2011	*Rattus rattus*
RodPV/Rattus_rattus/KF245958/Mayotte/2012/Murinae/Muridae	KF245958	Mayotte	2012	*Rattus rattus*
RodPV/Rattus_rattus/KF245960/Mayotte/2012/Murinae/Muridae	KF245960	Mayotte	2012	*Rattus rattus*
RodPV/Rattus_rattus/KF245963/Madagascar/2010/Murinae/Muridae	KF245963	Madagascar	2010	*Rattus rattus*
RodPV/Rattus_rattus/KF245968/Madagascar/2010/Murinae/Muridae	KF245968	Madagascar	2010	*Rattus rattus*
RodPV/Rattus_rattus/KF245969/Madagascar/2010/Murinae/Muridae	KF245969	Madagascar	2010	*Rattus rattus*
RodPV/Rattus_rattus/KF245976/Madagascar/2011/Murinae/Muridae	KF245976	Madagascar	2011	*Rattus rattus*

RodPV/Rattus_rattus/KF245990/Madagascar/2011/Murinae/Muridae	KF245990	Madagascar	2011	*Rattus rattus*
RodPV/Rattus_rattus/KF246002/Madagascar/2011/Murinae/Muridae	KF246002	Madagascar	2011	*Rattus rattus*
RodPV/Rattus_rattus/KF246003/Madagascar/2011/Murinae/Muridae	KF246003	Madagascar	2011	*Rattus rattus*
RodPV/Rattus_rattus/KF246004/Madagascar/2011/Murinae/Muridae	KF246004	Madagascar	2011	*Rattus rattus*
RodPV/Rattus_rattus/KF246005/Madagascar/2011/Murinae/Muridae	KF246005	Madagascar	2011	*Rattus rattus*
RodPV/Rattus_rattus/KF246014/Madagascar/2011/Murinae/Muridae	KF246014	Madagascar	2011	*Rattus rattus*
RodPV/Rattus_rattus/KF246023/Madagascar/2011/Murinae/Muridae	KF246023	Madagascar	2011	*Rattus rattus*
RodPV/Rattus_rattus/KF246025/Madagascar/2012/Murinae/Muridae	KF246025	Madagascar	2012	*Rattus rattus*
RodPV/Rattus_rattus/KF246026/Madagascar/2012/Murinae/Muridae	KF246026	Madagascar	2012	*Rattus rattus*
RodPV/Rattus_rattus/KF246027/Madagascar/2012/Murinae/Muridae	KF246027	Madagascar	2012	*Rattus rattus*
RodPV/Rattus_rattus/KF246029/Madagascar/2012/Murinae/Muridae	KF246029	Madagascar	2012	*Rattus rattus*
RodPV/Rattus_rattus/KF246034/Madagascar/2012/Murinae/Muridae	KF246034	Madagascar	2012	*Rattus rattus*
RodPV/Rattus_rattus/KF408256/Reunion/2012/Murinae/Muridae	KF408256	Reunion	2012	*Rattus rattus*
RodPV/Rattus_rattus/KF408260/Reunion/2012/Murinae/Muridae	KF408260	Reunion	2012	*Rattus rattus*
RodPV/Rattus_rattus/KJ408420/Tunisia/2013/Murinae/Muridae	KJ408420	Tunisia	2013	*Rattus rattus*
RodPV/Rattus_rattus/KJ408421/Tunisia/2013/Murinae/Muridae	KJ408421	Tunisia	2013	*Rattus rattus*
RodPV/Rattus_rattus/KJ408422/Tunisia/2013/Murinae/Muridae	KJ408422	Tunisia	2013	*Rattus rattus*
RodPV/Rattus_rattus/KJ408423/Tunisia/2013/Murinae/Muridae	KJ408423	Tunisia	2013	*Rattus rattus*
RodPV/Rattus_rattus/KJ408424/Tunisia/2013/Murinae/Muridae	KJ408424	Tunisia	2013	*Rattus rattus*
RodPV/Rattus_rattus/KJ408425/Tunisia/2013/Murinae/Muridae	KJ408425	Tunisia	2013	*Rattus rattus*
RodPV/Rattus_rattus/AB844426/Zambia/2010/Murinae/Muridae*	AB844426	Zambia	2010	*Rattus rattus*
RodPV/Rattus_rattus/AB844427/Zambia/2010/Murinae/Muridae	AB844427	Zambia	2010	*Rattus rattus*
RodPV/Rattus_Sp./KF245941/Reunion/2012/Murinae/Muridae	KF245941	Reunion	2012	*Rattus sp.*
RodPV/Rattus_Sp./KF245944/Reunion/2012/Murinae/Muridae	KF245944	Reunion	2012	*Rattus sp.*
RodPV/Rattus_Sp./KF245945/Reunion/2012/Murinae/Muridae	KF245945	Reunion	2012	*Rattus sp.*
RodPV/Rattus_Sp./KF245946/Reunion/2012/Murinae/Muridae	KF245946	Reunion	2012	*Rattus sp.*
RodPV/Rattus_Sp./KF245947/Reunion/2012/Murinae/Muridae	KF245947	Reunion	2012	*Rattus sp.*
RodPV/Rattus_SP./KF245949/Reunion/2012/Murinae/Muridae	KF245949	Reunion	2012	*Rattus sp.*
RodPV/Rattus_Sp./KF245952/Reunion/2012/Murinae/Muridae	KF245952	Reunion	2012	*Rattus sp.*
RodPV/Rattus_Sp./KF245957/Mayotte/2012/Murinae/Muridae	KF245957	Mayotte	2012	*Rattus sp.*
RodPV/Rattus_Sp./KF245959/Mayotte/2012/Murinae/Muridae	KF245959	Mayotte	2012	*Rattus sp.*
RodPV/Rattus_Sp./KF245961/Mayotte/2012/Murinae/Muridae	KF245961	Mayotte	2012	*Rattus sp.*
RodPV/Rhabdomys_pumilio/HQ660172/South_Africa/2009/Murinae/Muridae	HQ660172	South Africa	2009	*Rhabdomys pumilio*
RodPV/Rhabdomys_pumilio/HQ660173/South_Africa/2009/Murinae/Muridae	HQ660173	South Africa	2009	*Rhabdomys pumilio*
RodPV/Rhabdomys_pumilio/HQ660174/South_Africa/2009/Murinae/Muridae	HQ660174	South Africa	2009	*Rhabdomys pumilio*
RodPV/Rhabdomys_pumilio/HQ660175/South_Africa/2009/Murinae/Muridae	HQ660175	South Africa	2009	*Rhabdomys pumilio*
RodPV/Rhabdomys_pumilio/HQ660176/South_Africa/2009/Murinae/Muridae	HQ660176	South Africa	2009	*Rhabdomys pumilio*

RodPV/Rhabdomys_pumilio/HQ660177/South_Africa/2009/Murinae/Muridae	HQ660177	South Africa	2009	*Rhabdomys pumilio*
RodPV/Rhabdomys_pumilio/HQ660178/South_Africa/2009/Murinae/Muridae	HQ660178	South Africa	2009	*Rhabdomys pumilio*
RodPV/Rhabdomys_pumilio/HQ660179/South_Africa/2009/Murinae/Muridae	HQ660179	South Africa	2009	*Rhabdomys pumilio*
RodPV/Rhabdomys_pumilio/HQ660180/South_Africa/2009/Murinae/Muridae	HQ660180	South Africa	2009	*Rhabdomys pumilio*
RodPV/Microtus_agrestis/HQ660184/Germany/2010/Arvicolinae/Cricetidae	HQ660184	Germany	2010	*Microtus agrestis*
Tailam virus/Rattus_andamanensis/JN689227/China/2009/Murinae/Muridae	JN689227	China	2009	*Rattus andamanensis*

[a]: Virus name or virus affiliation/typical host/ accession number/origin/collection year/host Subfamily/host Family

[b]: RodPV = Rodents Paramyxovirus; sp. = species.

*: Two Respirovirus used as an outgroup.

130 **Supplementary Table S3:** Viruses used for the estimate of the evolutionary rate change for
131 the partial L-gene among the Measles virus.

Virus ID [a,b]	Accession numbers	Country	Collection year	Host [b]
Measles_virus/Human/FJ161211/China/2006	FJ16121	China	2006	*Human*
Measles_virus/ND/HM439386/Sudan/1997	HM439386	Sudan	1997	*ND*
Measles_virus/Human/JF727649/Russia/2010	JF727649	Russia	2010	*Human*
Measles_virus/Human/JF727650/Russia/2010	JF727650	Russia	2010	*Human*
Measles_virus/Human/GQ376026/Japan/1999	GQ376026	Japan	1999	*Human*
Measles_virus/Human/GQ376027/Japan/1999	GQ376027	Japan	1999	*Human*
Measles_virus/ND/AB481088/Japan/1989	AB481088	Japan	1989	*ND*
Measles_virus/ND/AB481087/Japan/1989	AB481087	Japan	1989	*ND*
Measles_virus/Human/KC164757/Italy/2010	KC164757	Italy	2010	*Human*
Measles_virus/ND/KJ018971/Canada/2010	KJ018971	Canada	2010	*ND*
Measles_virus/Human/KJ410048/Germany/2013	KJ410048	Germany	2013	*Human*
Measles_virus/Human/KC164758/Italy/2011	KC164758	Italy	2011	*Human*
Measles_virus/ND/KJ018970/Canada/2010	KJ018970	Canada	2010	*ND*
Measles_virus/ND/KM054581/France/2014	KM054581	France	2014	*ND*
Measles_virus/Human/JN635406/USA/2008	JN635406	USA	2008	*Human*
Measles_virus/Human/JN635410/USA/2003	JN635410	USA	2003	*Human*
Measles_virus/Human/JN635403/USA/2009	JN635403	USA	2009	*Human*
Measles_virus/Human/JN635408/USA/2005	JN635408	USA	2005	*Human*
Measles_virus/Human/JN635402/USA/2009	JN635402	USA	2009	*Human*
Measles_virus/Human/JN635411/USA/2009	JN635411	USA	2009	*Human*
Measles_virus/Human/JN635407/USA/2007	JN635407	USA	2007	*Human*
Measles_virus/Human/JN635404/USA/2009	JN635404	USA	2009	*Human*
Measles_virus/Human/JN635405/USA/2008	JN635405	USA	2008	*Human*
Measles_virus/Human/JN635409/USA/2004	JN635409	USA	2004	*Human*

132 [a]: Virus name/ typical host/ accession number/origin/collection year

133 [b]: ND = not defined.

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137 **Supplementary Table S4:** Significant diffusion rates between countries at Bayesian
138 phylogeographic analysis. Posterior probability of the rates k (pk) and Bayes factor (BF) of
139 the significant migration rates as revealed by the Skygrid symmetric model.

Rate (from/to)	pk	BF
Zambia / TrinidadTobago	0.98	207.27
Australia / Madagascar	0.97	193.02
Zambia / Madagascar	0.97	191.93
Tunisia / Madagascar	0.97	177.40
South_Africa / TrinidadTobago	0.97	160.21
South_Africa / Madagascar	0.97	146.70
TrinidadTobago / Tunisia	0.97	144.16
TrinidadTobago / Mayotte	0.96	127.91
Seychelles / Madagascar	0.96	123.41
Mayotte / Madagascar	0.966	119.23
Madagascar / China	0.96	117.75
TrinidadTobago / China	0.96	116.92
Germany / Madagascar	0.96	115.56
Reunion / TrinidadTobago	0.96	108.65
TrinidadTobago / Germany	0.96	102.95
Reunion / Madagascar	0.96	100.14
South_Africa / Tunisia	0.95	98.66
Tunisia / Mayotte	0.95	96.04
Seychelles / Mayotte	0.95	91.55
South_Africa / Australia	0.95	89.15
TrinidadTobago / Seychelles	0.94	75.17
South_Africa / Seychelles	0.94	73.71
Australia / Mayotte	0.94	70.02
Australia / China	0.93	60.21
Tunisia / China	0.93	57.20
Reunion / Tunisia	0.93	55.90
Zambia / China	0.92	49.00
Reunion / Australia	0.92	48.90
Seychelles / China	0.91	45.77
Seychelles / Germany	0.91	43.80
Australia / Germany	0.91	42.64

Reunion / Germany	0.90	41.77
Reunion / Seychelles	0.90	41.06
Zambia / Reunion	0.90	37.85
TrinidadTobago / Australia	0.89	37.21
Tunisia / Germany	0.89	34.10
Zambia / Germany	0.88	31.14
Germany / Mayotte	0.88	30.75
South_Africa / Germany	0.87	29.01
Reunion / Mayotte	0.87	28.80
Mayotte / China	0.87	28.31
Germany / China	0.85	25.38
Reunion / China	0.83	21.52
South_Africa / China	0.83	20.66
South_Africa / Reunion	0.80	17.01
TrinidadTobago / Madagascar	0.79	15.81

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Annexe 6. Serological evidence of widespread circulation of single stranded RNA viruses in wild fauna from the South Western Indian Ocean islands.

(En cours de soumission)

Séroprévalence obtenue pour 643 chauves-souris et 256 petits mammifères terrestres de la zone SOOI par la technique d'indirect immunofluorescence assay (IIFA) en utilisant un panel de virus à ARN simple brin appartenant à 5 familles et 10 genres virales.

Matériels et Méthodes

Collecte des individus

De Mars 2010 à Mars 2015, 643 chauves-souris ont été collectés à Mayotte (archipel des Comores), La Réunion, Maurice (Mascareignes archipel), Madagascar et Mahé (archipel des Seychelles). Ces individus se répartissaient en 7 familles, 11 genres et 30 espèces dont 4 espèces frugivores et 26 espèces insectivores. D'autre part, 256 petits mammifères terrestres ont été collectés à Mayotte (archipel des Comores), La Réunion Maurice (Mascareignes archipel), Madagascar et Mahé (archipel des Seychelles) et qui se répartissaient en 2 familles, 3 genres et 5 espèces.

Détection d'anticorps

La détection des anticorps a été réalisée en utilisant les lames Euroimmun Mosaïque Chip (EU38, Lübeck, Allemagne). Les lames présentent sur des champs séparés, des cellules infectées au niveau de chaque champ par un virus ARN donné (avec en tout 15 virus analysables) représentant 5 familles virales, et 10 genres :

Genre *Coronavirus* : *SARS coronavirus*, (SRAS; UE 14, souche Francfort-1) ; *Human coronavirus* (HCoV-229E ; UE 169, souche ATCC (VR-740).

Genre *Flavivirus* : *West Nile virus* (VNO; (UE 14, souche Ouganda) ; *Yellow Fever virus* (YFV; UE 14, souche vaccinale RKI-17D); *Dengue virus* (DENV-2; UE 14, souche TH-36).

Genre *Hantavirus* : DOBV (DOBVII; UE 14, souche DOB / Slov).

Genre *Phlébovirus :* RFVF (RFVF; UE 14, souche du Rift 12 / 2/01).

Genre *Nairovirus :* CCHF (CCHFV; UE 90 Zellen, glycoprotéine exprimée Gc (GPC-Protein).

Genre *Morbillivirus* : *Measles* (UE 38, souche Edmonston).

Genre *Rubulavirus* : *Mumps* (UE 38, souche Jones) ; *PIV-2*(PIVII; UE 18/9, souche Greer).

Genre *Avulavirus* : PIV-1(PIV-I; UE 18/9, souche Sendai CPJ-3)

Genre *Pneumovirus* : RSV (RSV; UE 38, souche RSV B Wash / 18537 / '62 (CH 18537);

Genre *Alphavirus* : CHIKV (CHIKV; UE 14, souche OPY1, LR2006; La *Réunion 2006*); VEEV (VEEV; UE 14, souche vaccinale).

La première incubation s'est faite par ajout de 70µL de sérum d'un individu dilué au 1/40 sur une puce. L'incubation s'est fait à température ambiante pendant 2 heures. Les lames sont ensuite rincées en Phosphate Buffer Saline (PBS) + 0.1% Tween (PBS-T) pendant 5 min et séchées à l'air libre. La seconde incubation s'est faite par ajout d'immunoglobuline (Ig) de chèvre anti-chauves-souris ou anti-souris (Bethyl Laboratories, Montgomery, TX, USA) dilué au 1/1000 dans du tampon Euroimmun. Les lames sont ensuite rincées en PBS-T pendant 5 min. La troisième incubation s'est faite par ajout d'Ig de singe anti-chèvre conjugué à la cyanine 3 (Dianova, Hamburg, Germany) dilué au 1/2 en glycérol puis au 1/50 dans du PBS et incubés pendant 30 minutes à température ambiante. Les lames sont ensuite rincées en PBS-T pendant 10 minutes et séchées. Les noyaux cellulaires sont colorés au 4′,6-diamidino-2-phenylindole (DAPI) et les lames recouvertes d'une lamelle. La lecture s'est faite au microscope à fluorescence (Zeiss).

Résultats

Réactivités sérologique des chauves-souris et petits mammifères terrestres de la zone SOOI testés par IIFA contre 5 familles dont 10 genres viraux. Résultats exprimés en nombres d'individus positifs sur nombre d'individus total testé. Pourcentage de séropositivité indiqué entre parenthèse.

A. Coronaviridae

		Coronavirus	
Famille	**Espèce**	**SARS-CoV**	**HCoV-229E**
Emballuronidae	*Emballonura tiavato*	0/1	0/1
Hipposideridae	*Hipposideros commersoni*	0/14	0/14
Miniopteridae	*Miniopterus aelleni*	0/5	0/5
	Miniopterus cf. *ambohitrensis*	0/30	2/30 (6.7)
	Miniopterus giffithsi	1/6 (16.7)	0/6
	Miniopterus gleni	0/13	4/13 (30.8)
	Miniopterus griveaudi	1/38 (2.7)	1/38 (2.6)
	Miniopterus mahafaliensis	0/38	0/38
	Miniopterus sororculus	0/8	0/8
Molossidae	*Chaerephon atsinanana*	0/7	1/7 (14.3)
	Chaerephon leucogaster	1/43 (2.3)	1/43 (2.3)
	Mops leucostigma	0/32	0/32
	Mops midas	0/16	0/16
	Mormopterus acetabulosus	2/36 (5.6)	0/36
	Mormopterus francoismoutoui	0/46	2/46 (4.3)
	Mormopterus jugularis	3/73 (4.1)	2/73 (2.7)
	Otomops madagascariensis	1/31 (3.2)	0/31
Pteropodidae	*Eidolon dupreanum*	0/11	0/11
	Pteropus niger	0/24	0/24
	Pteropus rufus	0/17	0/17
	Pteropus seychelliensis	1/39 (2.6)	1/39 (2.6)
	Rousettus madagascariensis	1/42 (2.4)	2/42 (4.8)
Rhinonycteridae	*Triaenops furculus*	0/6	0/6
	Triaenops menamena	0/32	2/32 (6.25)
Vespertilionidae	*Myotis goudoti*	2/28 (7.1)	1/28 (3.6)
	Neoromicia	0/2	0/2
	Pipistrellus raceyi	0/1	0/1
	Scotophilus marovaza	0/1	0/1
	vesper	0/3	0/3
Chauves-souris		13/643 (2.0)	19/643 (2.9)
Muridae	*Mus musculus*	0/3	1/3 (33.3)
	Rattus norvegicus	5/41 (12.2)	1/41 (2.4)
	Rattus rattus	0/155	1/155 (0.6)
Nesomyidae	*Eliurus majori*	0/4	1/4 (25)
	Eliurus minor	2/53 (3.8%)	0/53 (0%)
Mammifères terrestres		7/256 (2.7)	4/256 (1.6)

B. Flaviviridae

		Flavivirus		
Famille	**Espèce**	**WNV**	**YFV**	**DENV-2**
Emballuronidae	*Emballonura tiavato*	0/1	0/1	0/1
Hipposideridae	*Hipposideros commersoni*	0/14	0/14	0/14
Miniopteridae	*Miniopterus aelleni*	1/5 (20)	0/5	1/5 (20.0)
	Miniopterus cf. *ambohitrensis*	4/30 (13.3)	13/30 (43.3)	10/30 (33.3)
	Miniopterus giffithsi	0/6	1/6 (16.7)	0/6
	Miniopterus gleni	2/13 (15.4)	3/13 (23.1)	3/13 (23.1)
	Miniopterus griveaudi	3/38 (7.9)	2/38 (5.3)	1/38 (2.6)
	Miniopterus mahafaliensis	0/38	4/38 (10.5)	5/38 (13.2)
	Miniopterus sororculus	0/8 ()	3/8 (37.5)	2/8 (25.0)
Molossidae	*Chaerephon atsinanana*	1/7 (14.3)	1/7 (14.3)	1/7 (14.3)
	Chaerephon leucogaster	7/43 (16.3)	12/43 (27.9)	10/43 (23.3)
	Mops leucostigma	0/32	1/32 (3.1)	1/32 (3.0)
	Mops midas	0/16	1/16 (63)	1/16 (6.3)
	Mormopterus acetabulosus	0/36	4/36 (11.1)	5/36 (13.9)
	Mormopterus francoismoutoui	1/46 (2.2)	9/46 (19.6)	10/46 (21.7)
	Mormopterus jugularis	14/73 (19.2)	45/73 (61.6)	35/73 (47.9)
	Otomops madagascariensis	3/31 (9.7)	8/31 (25.8)	6/31 (19.4)
Pteropodidae	*Eidolon dupreanum*	9/11 (81.8)	11/11 (100)	10/11 (90.9)
	Pteropus niger	1/24 (4.7)	2/24 (8.3)	3/24 (12.5)
	Pteropus rufus	5/17 (29.4)	5/17 (29.4)	6/17 (35.3)
	Pteropus seychelliensis	3/39 (7.7)	11/39 (28.2)	11/39 (28.2)
	Rousettus madagascariensis	2/42 (4.8)	4/42 (9.5)	5/42 (11.9)
Rhinonycteridae	*Triaenops furculus*	0/6	1/6 (16.7)	0/6
	Triaenops menamena	1/32 (3.125)	1/32 (3.125)	0/32
Vespertilionidae	*Myotis goudoti*	1/28 (3.6)	1/28 (3.6)	1/28 (3.6)
	Neoromicia	0/2	0/2	0/2
	Pipistrellus raceyi	0/1	0/1	0/1
	Scotophilus marovaza	0/1	0/1	0/1
	vesper	0/3	0/3	0/3
Chauves-souris		58/643 (9.0)	143/643 (22.2)	127/643 (19.6)
Muridae	*Mus musculus*	0/3	0/3	0/3
	Rattus norvegicus	0/41	1/41 (2.4)	5/41 (12.2)
	Rattus rattus	5/155 (3.2)	52/155 (33.5)	36/155 (23.2)
Nesomyidae	*Eliurus majori*	0/4	0/4	0/4
	Eliurus minor	0/53 (0%)	0/53(0%)	1/53 (1.9%)
Mammifères terrestres		5/256 (1.9)	53/256 (20.7)	42/256 (16.4)

C. Bunyaviridae

		Hantavirus	***Phlébovirus***	***Nairovirus***
Famille	**Espèce**	**DOBV**	**RVFV**	**CCHFV**
Emballuronidae	*Emballonura tiavato*	0/1	0/1	0/1
Hipposideridae	*Hipposideros commersoni*	0/14	0/14	1/14 (7.1)
Miniopteridae	*Miniopterus aelleni*	0/5	0/5	1/5 (20)
	Miniopterus cf. *ambohitrensis*	0/30	0/30	5/30 (16.7)
	Miniopterus giffithsi	0/6	0/6	2/6 (33.3)
	Miniopterus gleni	0/13	0/13	2/13 (15.4)
	Miniopterus griveaudi	1/38 (2.6)	0/38	7/38 (18.4)
	Miniopterus mahafaliensis	0/38	0/38	4/38 (10.5)
	Miniopterus sororculus	0/8 ()	0/8 ()	1/8 (12.5)
Molossidae	*Chaerephon atsinanana*	0/7	0/7	0/7
	Chaerephon leucogaster	1/43 (2.3)	1/43 (2.3)	2/43 (4.7)
	Mops leucostigma	0/32	3/32 (9.4)	1/32 (3.1)
	Mops midas	0/16	0/16	0/16
	Mormopterus acetabulosus	0/36	1/36 (2.8)	2/36 (5.6)
	Mormopterus francoismoutoui	0/46	0/46	0/46
	Mormopterus jugularis	0/73	5/73 (6.8)	5/73 (6.8)
	Otomops madagascariensis	0/31	2/31 (6.5)	15/31 (48.4)
Pteropodidae	*Eidolon dupreanum*	0/11	2/11 (18.2)	2/11 (18.2)
	Pteropus niger	2/24 (8.3)	3/24 (12.5)	0/24
	Pteropus rufus	1/17 (5.9)	1/17 (5.9)	0/17
	Pteropus seychelliensis	12/39 (30.8)	17/39 (43.6)	5/39 (12.8)
	Rousettus madagascariensis	0/42	0/42	1/42 (2.4)
Rhinonycteridae	*Triaenops furculus*	0/6	0/6	0/6
	Triaenops menamena	0/32	0/32	2/32 (6.25)
Vespertilionidae	*Myotis goudoti*	0/28	0/28	2/28 (7.1)
	Neoromicia	0/2	0/2	0/2
	Pipistrellus raceyi	0/1	0/1	0/1
	Scotophilus marovaza	0/1	0/1	0/1
	vesper	0/3	0/3	0/3
Chauves-souris		17/643 (2.6)	35/643 (5.4)	60/643 (9.3)
Muridae	*Mus musculus*	0/3	0/3	1/3 (33.3)
	Rattus norvegicus	3/41 (7.3)	5/41 (12.2)	2/41 (4.9)
	Rattus rattus	33/155 (21.2)	45/155 (29.0)	22/155 (14.2)
Nesomyidae	*Eliurus majori*	0/4	0/4	0/4
	Eliurus minor	1/53 (1.9%)	3/53 (5.7%)	3/53 (5.7%)
Mammifères terrestres		37/256 (14.5)	53/256 (20.7)	28/256 (10.9)

D. *Paramyxoviridae*

		Morbillivirus	***Rubulavirus***		***Pneumovirus***	***Respirovirus***
Famille	**Espèce**	**Measles**	**Mumps**	**PIV-2**	**RSV**	**PIV-1**
Emballuronidae	*Emballonura tiavato*	0/1	0/1	0/1	0/1	0/1
Hipposideridae	*Hipposideros commersoni*	3/14 (21.4)	4/14 (28.6)	4/14 (28.6)	1/14 (7.1)	2/14 (14.3)
Miniopteridae	*Miniopterus aelleni*	1/5 (20.0)	0/5	1/5 (20.0)	1/5 (20.0)	0/5
	Miniopterus cf. *ambohitrensis*	6/30 (20.0)	12/30 (40.0)	9/30 (30.0)	8/30 (26.7)	1/30 (3.3)
	Miniopterus giffithsi	0/6	1/6 (16.7)	3/6 (50.0)	0/6	0/6
	Miniopterus gleni	1/13 (7.7)	1/13 (7.7)	2/13 (15.4)	0/13	3/13 (23.1)
	Miniopterus griveaudi	8/38 (21.1)	11/38 (28.9)	8/38 (21.1)	4/38 (10.5)	7/38 (18.4)
	Miniopterus mahafaliensis	5/38 (13.2)	10/38 (26.3)	4/38 (10.5)	1/38 (2.6)	3/38 (7.9)
	Miniopterus sororculus	2/8 (25.0)	4/8 (50.0)	4/8 (50.0)	2/8 (25.0)	3/8 (37.5)
Molossidae	*Chaerephon atsinanana*	0/7	0/7	2/7 (28.6)	0/7	1/7 (14.3)
	Chaerephon leucogaster	11/43 (25.6)	11/43 (25.6)	9/43 (20.9)	4/43 (9.3)	1/43 (2.3)
	Mops leucostigma	1/32 (3.1)	1/32 (3.1)	5/32 (15.6)	0/32	3/32 (9.4)
	Mops midas	5/16 (31.3)	2/16 (12.5)	3/16 (18.75)	1/16 (6.3)	0/16
	Mormopterus acetabulosus	0/36	7/36 (19.4)	2/36 (5.6)	0/36	3/36 (8.3)
	Mormopterus francoismoutoui	0/46	5/46 (10.9)	4/46 (8.7)	1/46 (2.1)	4/46 (8.7)
	Mormopterus jugularis	16/73 (21.9)	38/73 (52.1)	21/73 (28.8)	3/73 (4.1)	2/73 (2.7)
	Otomops madagascariensis	10/31 (32.3)	9/31 (29.0)	5/31 (16.1)	5/31 (16.1)	5/31 (16.1)
Pteropodidae	*Eidolon dupreanum*	0/11	7/11 (63.6)	8/11 (72.7)	0/11	0/11
	Pteropus niger	1/24 (4.7)	3/24 (12.5)	15/24 (62.5)	0/24	1/24 (4.2)
	Pteropus rufus	0/17	3/17 (17.6)	11/17 (64.7)	0/17	0/17
	Pteropus seychelliensis	2/39 (5.1)	12/39 (30.8)	12/39 (30.8)	2/39 (5.1)	0/39
	Rousettus madagascariensis	3/42 (7.1)	19/42 (45.2)	25/42 (59.5)	2/42 (4.7)	3/42 (7.1)
Rhinonycteridae	*Triaenops furculus*	1/6 (16.7)	1/6 (16.7)	0/6	0/6	0/6
	Triaenops menamena	4/32 (12.5)	2/32 (6.25)	6/32 (18.8)	3/32 (9.4)	2/32 (6.3)
Vespertilionidae	*Myotis goudoti*	5/28 (17.9)	6/28 (21.4)	7/28 (25.0)	2/28 (7.1)	3/28 (10.7)
	Neoromicia	1/2 (50)	1/2 (50)	0/2	0/2	0/2
	Pipistrellus raceyi	0/1	0/1	0/1	0/1	0/1
	Scotophilus marovaza	0/1	0/1	0/1	0/1	0/1
	vesper	0/3	0/3	0/3	0/3	0/3
Chauves-souris		86/643 (13.4)	170/643 (26.4)	170/643 (26.4)	40/643 (6.2)	47/643 (7.3)
Muridae	*Mus musculus*	2/3 (66.7)	2/3 (66.7)	3/3(100)	2/3(66.7)	0/3
	Rattus norvegicus	13/41 (31.7)	16/41 (39.0)	9/41(21.9)	7/41(17.1)	4/41(9.8)
	Rattus rattus	34/155 (21.9)	58/155 (37.4)	46/155(29.7)	26/155(16.8)	47/155(30.3)
Nesomyidae	*Eliurus majori*	0/4	0/4	0/4	0/4	1/4(25)
	Eliurus minor	16/53 (30.2%)	15/53 (28.3%)	6/53 (11.3%)	6/53 (11.3%)	7/53 (13.2%)
Mammifères terrestres		65/256 (25.4)	91/256 (35.5)	64/256(25.0)	41/256(16.0)	59/256(23.0)

E. *Togaviridae*

		Alphavirus	
Famille	**Espèce**	**VEEV**	**CHIKV**
Emballuronidae	*Emballonura tiavato*	0/1	0/1
Hipposideridae	*Hipposideros commersoni*	0/14	0/14
Miniopteridae	*Miniopterus aelleni*	0/5	0/5
	Miniopterus cf. *ambohitrensis*	0/30	1/30 (3.3)
	Miniopterus giffithsi	0/6	0/6
	Miniopterus gleni	1/13 (7.7)	1/13 (7.7)
	Miniopterus griveaudi	0/38	0/38
	Miniopterus mahafaliensis	0/38	0/38
	Miniopterus sororculus	0/8 ()	1/8 (12.5)
Molossidae	*Chaerephon atsinanana*	0/7	1/7 (14.3)
	Chaerephon leucogaster	0/43	0/43
	Mops leucostigma	0/32	0/32
	Mops midas	0/16	0/16
	Mormopterus acetabulosus	0/36	0/36
	Mormopterus francoismoutoui	0/46	0/46
	Mormopterus jugularis	0/73	1/73 (1.4)
	Otomops madagascariensis	0/31	5/31 (16.1)
Pteropodidae	*Eidolon dupreanum*	0/11	0/11
	Pteropus niger	0/24	1/24 (4.7)
	Pteropus rufus	0/17	0/17
	Pteropus seychelliensis	0/39	7/39 (17.9)
	Rousettus madagascariensis	0/42	1/42 (2.4)
Rhinonycteridae	*Triaenops furculus*	0/6	0/6
	Triaenops menamena	0/32	0/32
Vespertilionidae	*Myotis goudoti*	1/28 (3.6)	1/28 (3.6)
	Neoromicia	0/2	0/2
	Pipistrellus raceyi	0/1	0/1
	Scotophilus marovaza	0/1	0/1
	vesper	0/3	0/3
Chauves-souris		2/643 (0.3)	20/643 (3.1)
Muridae	*Mus musculus*	0/3	0/3
	Rattus norvegicus	0/41	3/41 (7.3)
	Rattus rattus	0/155	10/155 (6.5)
Nesomyidae	*Eliurus majori*	0/4	/4
	Eliurus minor	0/53 (0%)	3/53 (5.7%)
Mammifères terrestres		0/256	0/256 (6.3)

Annexe 7. Liste des publications

Contribution à d'autres travaux: publication acceptée

1. Salez, N., **Mélade, J**., Pascalis, H., Aherfi, S., Dellagi, K., Charrel, RN., Carrat, F. & de Lamballerie, X. (2014). Influenza C virus high seroprevalence rates observed in 3 different population groups. J. Infect. Aug;69(2):182-9. doi: 10.1016/j.jinf.2014.03.016. Epub 2014 Apr 4.

Faisant partie du sujet de thèse : publication acceptée

2. Wilkinson, D.A., **Mélade, J**., Dietrich, M., Ramasindrazana, B., Soarimalala, V., Lagadec, E., le Minter, G., Tortosa, P., Heraud, J.M., de Lamballerie, X., Goodman, S.M., Dellagi, K. & Pascalis, H**.** (2014). Highly diverse morbillivirus-related paramyxoviruses in wild fauna of the southwestern Indian Ocean Islands: evidence of exchange between introduced and endemic small mammals. J. Virol. Aug;88(15):8268-77. doi: 10.1128/JVI.01211-14. Epub 2014 May 14.

Faisant partie du sujet de thèse : publications soumises

3. **Mélade, J**., McCulloch, S., Ramasindrazana, B., Lagadec, E., Turpin, M., Pascalis, H., Goodman, S.M., Markotter, W. & Dellagi, K. Serological evidence of diverse circulation of lyssaviruses among bats on southwestern Indian Ocean islands (en revision).

4. **Mélade, J**., Wieseke, N., Ramazindrazana, B., Flores, O., Lagadec, E., Gommard, Y., Goodman, S.M., Dellagi, K., & Pascalis, H. An eco-epidemiological study of Morbilli-related paramyxovirus infection in Madagascar bats reveals host-switching as the dominant macro-evolutionary mechanism (en revision).

Faisant partie du sujet de thèse : publication en cours de soumission

5. **Mélade, J**., Müller, M., Ramasindrazana, B., Lagadec, E., Turpin, M., Pascalis, H., Goodman, S.M., Drosten, C. & Dellagi, K. Serological evidence of widespread circulation of single stranded RNA viruses in wild fauna from the South Western Indian Ocean islands (en preparation).

En rapport avec le sujet de thèse : publication soumise

6. Ghawar, W., Pascalis, H., Bettaie, J., Wilkinson, D., **Mélade, J**., El Gharbi, A., Snoussi, M.A., Laouini, D., Ben Salah, A. & Dellagi, K. Insight into the global evolution of Rodentia associated Morbillirelated Paramyxoviruses reveals the role of Rattus as a worldwide spreader (en revision).

Annexe 8. Formations, congrès

1. Congrès international « **Bats, small mammals and infectious agents** » (2013, St Denis, La Réunion). Communication orale.

2. Congrès international « **African Small Mammal Symposium »** (2015, Mantasoa, Madagascar). Communication orale.

3. Journées scientifiques de la « **Fédération Environnement, Biodiversité et Santé** » (2014, Saint-Denis La Réunion). Communication orale.

4. Colloque **FEDER POCT Biodiversité** (2015, Saint-Denis La Réunion). Communication orale.

5. Stage doctoral de 3 semaines du 6 Septembre 2014 au 27 Septembre 2014 au Centre Hospitalier Universitaire de Bonn en Allemagne dans le laboratoire de Virologie du Pr Christian Drosten.

6. Stage doctoral de 5 mois à l'Université de Pretoria en Afrique du Sud dans le laboratoire de virologie du Pr Wanda Markotter.

Résumé

La faune sauvage a été depuis longtemps incriminée dans la survenue de zoonoses et joue le rôle de réservoir d'agents pathogènes (virus *Nipah*, *Hendra*, *Ebola*, *Hantaan* etc.) pour l'homme. Les îles tropicales et subtropicales du Sud-Ouest de l'Océan Indien (SOOI) constituent l'une des 34 régions reconnues comme « *hotspot* » de biodiversité au niveau mondial. Elles sont caractérisées par un très fort endémisme de la faune sauvage surtout sur l'Ile de Madagascar. Le caractère multi-insulaire de la région du SOOI, la diversité de ses biotopes et ses disparités biogéographiques et humaines offrent un champ d'investigation unique pour explorer « *in natura* » la dynamique évolutive des agents infectieux et les relations hôtes-virus.

Nos travaux de recherche ont porté sur deux modèles de virus à ARN de polarité négative, les paramyxovirus et les lyssavirus. Le premier modèle viral nous a permis d'aborder les questions relatives à la dynamique de transmission virale au sein de communauté d'hôtes, plus particulièrement, les chauves-souris et les petits mammifères terrestres de Madagascar et d'identifier les facteurs agissant sur cette dynamique de transmission et de diversification virale, en particulier les facteurs bio-écologiques associés à leurs hôtes. Le second modèle viral, les lyssavirus, nous a permis de décrire sur l'ensemble des îles du SOOI, la circulation virale dans ce système multi-insulaire diversifié, au sein des chauves-souris dont la plupart des espèces sont endémiques à cette région.

Dans l'ensemble, nos investigations ont permis de mettre en évidence des échanges viraux (« *host-switch* ») importants entre chauves-souris, petits mammifères terrestres endémiques de Madagascar et les rongeurs introduits, le rôle de ces mammifères en tant que réservoir viral majeur et souligner le rôle disséminateur de *Rattus rattus*. Par ailleurs, nous avons pu identifier ce phénomène de « *host-switch* » comme étant le mécanisme macro-évolutif prépondérant et l'importance des facteurs biotiques et abiotiques à l'origine de la dynamique de transmission et de la diversification virale observée chez les paramyxovirus de chauves-souris de Madagascar.

Mots-clés : faune sauvage, zoonoses, SOOI, biodiversité, lyssavirus, paramyxovirus, diversité virale, réservoir viral, disséminateur viral, « *host-switch* » facteurs écologique